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Evaluation of Fusarium moniliforme ear rot resistance in opaque-2 maize and interaction with various kernel characters

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Evaluation of *Fusarium moniliforme* ear rot resistance in opaque-2 maize and interaction with various kernel characters

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INTRODUCTION

Ear rots of maize can be a problem to farmers wherever maize is grown. Ear rots of maize reduce the yield and quality of the grain, and may decrease the germination of seed (21, 142). During the years 1916-1933, about one-fourth of the grain reaching U.S. terminal markets was graded as number three or poorer (greater than 6% damaged kernels). In years when weather conditions were favorable for ear rot development, almost 70% of the grain was graded as number three or poorer (53, 156). This situation has improved greatly since 1933, as Koehler (65) reported an average ear rot damage of only 3.5% from 1940-1951 in Illinois test plots. In developing countries, ear rots are still of importance (107).

Maize constitutes roughly 50-60% of the human dietary protein in some developing countries (10). Unfortunately maize is not a balanced source of protein for humans or single-stomached animals because of the low concentration of two essential amino acids, lysine and tryptophan (16). In 1964, when Mertz et al. (86) announced that the opaque-2 gene greatly improved the protein quality of maize by increasing the lysine and tryptophan content, it was believed that the incorporation of the opaque-2 gene into commercial maize was the ideal solution to rectify this amino acid imbalance. Coupled with the improved protein quality of opaque-2 maize, however, were several associated liabilities, which have

severely limited the use of opaque-2 maize. These included reduced yield, non-acceptibility of the grain phenotype, dry-milling problems, and greater susceptibility to insects and ear rotting pathogens, when compared to normal endosperm maize (152). Through breeding and selection, these undesirable traits have been diminished. Ear rots, however, still present a serious problem for opaque-2 maize.

The objectives of this study were:

(1) To determine the principle fungi responsible for ear rots of opaque-2 maize.

(2) To evaluate the methods of artificial inoculation and rating of ear rot for effective differentiation of resistant and susceptible genotypes.

(3) To test some of the existing opaque-2 germplasm in a mating design experiment to determine the combining abilities of public opaqe-2 inbreds for resistance to ear rot.

(4) To investigate the interaction between ear rot resistance and various kernel characteristics.

(5) To determine if Fusarium moniliforme-infected kernels result in systemic infection and subsequent ear rot.

LITERATURE REVIEW

Ear rots of maize may occur wherever maize is grown. Losses attributable to ear rotting fungi vary from negligible to substantial and are influenced by many factors, including weather, soil fertility, sanitation, crop rotation, insect feeding, seed health, and maize genotype. Numerous fungi are responsible for ear rots and their prevalence and destructiveness vary with geographical locale and weather conditions. Some of the more important fungi causing ear rots in the United States include Fusarium moniliforme Sheld., Diplodia zeae (Schw.) Lev., and Gibberella zeae (Schw.) Petch. Others include Nigrospora oryzae (B. & Br.) Petch, Physalospora zeae Stout, Penicillium sp., Helminthosporium sp., Phizopus sp., and Aspergillus sp. (65).

Fusarium moniliforme Ear Rot of Maize

Fusarium moniliforme is a world-wide pathogen of maize, ranging from the humid and semi-arid temperate zones to the sub-tropical and tropical areas of the globe (7, 107, 121). It is less common in the cooler temperate regions. In North America, it has been reported from the Gulf Coast (31) to southern Canada (156), and from New Jersey (92) to California (139). In most surveys, F. moniliforme has been found to be the predominant fungus associated with ear rots (8, 31, 32,

33, 50, 66, 81, 87) reaching 95-100% infection with some seed lots (37, 69, 87). Its prevalence, however, has been noted to vary with the region and year (32, 53, 87, 114). In addition to inciting ear rot of maize, it is also responsible for seedling blight, scutellum rot, root rot, and stalk rot (7, 65). The fungus also attacks several other members of the Gramineae, including rice, sorghum, and sugar cane (7, 163), and is responsible for seedling blight, root rot, stunting, and hypertrophy on members of 31 divergent plant families (7).

Economic significance

Economic losses attributable to Fusarium ear rot can be substantial. Unfortunately most of the surveys assessing ear rot incidence were made in the first half of the century when maize genotypes and cultural practices were vastly different from today. Results of these surveys are not comparable because infection incidence was estimated by three different methods: (i) ear rot based on ear examination, (ii) examination of shelled grain, or (iii) the percentage of carloads of various grades received at terminal markets (52, 66, 142). During the period from 1916-1941, the percentage of carloads of grain graded at more than 5% damage ranged from 1-90%, but this included rots due to all fungi. Based on whole ear examination, ear rot damage by F. moniliforme during the years

1924-1941 ranged from 0.5-8.5% (average 3.4%). Total ear rot damage caused by all fungi ranged from 3-15%. Based on examination of shelled grain from 1940-1951, damage due to F. moniliforme ranged from 0.5-2.5% (average 1.2%). In 1959, Koehler (65) conservatively estimated that ear rots caused an annual monetary loss of $1,500,000 in Illinois alone.

Aside from direct yield reduction due to ear rotting, the use of F. moniliforme infected seed may result in reduced germination with accompanying stand and yield reduction. Evidence for this is rather contradictory. Several researchers (65, 150) have the opinion that the fungus was not a vigorous pathogen and found no correlation between infection, and seedling blight, stand, or grain yield (9, 31, 85, 133). Other workers (28, 50, 55, 92, 153, 141) found the use of infected seed, as revealed by germination tests, significantly reduced the stand and subsequent yield. Part of the reason for the disparity of the findings may be due to the variability of the fungus, the influence of weather, and also the antagonism of soil organisms towards surface-borne F. moniliforme (92, 133, 72).

Grain infected with F. moniliforme can deteriorate further in storage and possibly become toxic to animals (21, 113). Under normal storage conditions, F. moniliforme is not a serious problem, but if grain moisture exceeds 23%, it will proliferate and often predominate, even under modified atmos-

pheric conditions (51, 146, 158). Fusarium-infected kernels are lower in nutritive value and palatability (89) and as early as 1904 it was noted that animals ingesting Fusarium infected maize developed symptoms resembling ergotism (113). F. moniliforme has since been shown to produce several heat-stable, water-soluble mycotoxins, including zearalenone and moniliformin (11, 21, 127).

Symptoms

Fusarium-ear rot is usually characterized by salmon pink to reddish brown discolored kernels scattered randomly over the ear (62, 64, 65, 81). As the disease progresses, cottony-pink mycelium develops on the infected kernels and rotted areas may coalesce. Rot may occur at the tip only following bird damage, around channels made by corn borers and other insects, and in silk-cut kernels (66, 138, 156, 159). Infected kernels may exhibit white streaks caused by air channels beneath the pericarp (62, 81). Many researchers (8, 87, 120, 141) have noted that a high percentage of apparently healthy seed are infected with F. moniliforme.

Etiology

Fungal morphology Fusarium moniliforme was first described by Sheldon in Nebraska in 1904 (132). It is the conidial or imperfect stage of the ascomycete Gibberella fujikuroi (Saw.) Wr. (syn. G. moniliforme (Sheld.) Snyd. &

Hans.). Morphology and culture characteristics as described by Booth (7) are:

> "Growth initially rather filmy, colorless, and rapid. Cultures from below typically dark violet but occasionally paler, lilac, vinaceous or even cream. Aerial mycelium is generally dense and delicately floccose to felted, white vinaceous to felted often with a powdered appearance due to the formation of microconidia. Microconidiophores are simple, lateral, subulate phialides formed on the aerial hyphae; rarely they may be formed on short lateral branches. They are 20-30 microns long by 2-3 microns at the base narrowing to approxiamtely 1.5 at the apex.
>
> Microconidia are formed in chains under optimum growing conditions and these can readily be observed in situ under the low power of the microscope. Microconidia measure 5-12 by 1.5-2.5 microns and are fusiform to clavate with a slightly flattened base; they occasionally become 1 septate.
>
> Macrocondial formation is rare in many strains; where present they develop from conidiophores formed as lateral branches on the hyphae. The conidiophore may consist of a single basal cell bearing 2-3 apical phialides or it may form 2-3 metulae which in trun bear simple dcliform to obclavate phialides. These measure 20-24 x 3.5-4 microns. Subeffuse sporodochia develop occasionally in some strains.
>
> Macroconidia are inequilaterally fusoid, delicate, thin-walled, with an elongated, often sharply curved apical cell and pedicellate basal cell. They are 3-7 septate, and measure 25-60 by 2.5-4 microns.
>
> Chlamydospores are absent in both mycelium and conidia. Swollen stromatic initial cells are frequently found and should not be confused with chlamydospores; these aggregate into dark blue irregularly globose sclerotia which may form the perithecial initials.
>
> Perithecia [rarely observed in the U.S.] occur only on dead plant material. They are superficial, dark blue, globose to conical, 250-350 microns high by 220-300 microns diam. and with a rough outer wall. Asci are ellipsoid to clavate with 4-8 obliquely uniserate to biserate ascospores. Ascospores are hyaline, ellipsoid, often remaining 1 septate but occasionally becoming 3 septate; they are 14-18 x 4.5-6 microns."

The variety F. moniliforme var. subglutinans Wr. & Reink. (conidial stage of G. fujikuroi var. subglutinans Edwards), often confused with F. moniliforme, is distinguished by microconidial formation on polyphialides and not in chains. Macroconidia are somewhat smaller (3-5 septate, 32-53 x 3-4.5 microns) and ascospores are narrower (12-15 x 4.5-5 microns). F. moniliforme var. subglutinans also attacks maize and the symptoms produced are indistinguishable from those caused by F. moniliforme (7).

Pathogenicity After Sheldon initially ascertained the pathogenicity of F. moniliforme and its role with ear rots of maize, many workers questioned its pathogenicity, especially as a seedling-blight pathogen. Holbert et al. (50), Moore (92) and Valleau (150) considered the fungus an active parasite, while Branstetter (9), Koehler (65), and Melchers and Johnston (85) believed it was a weak or secondary pathogen. Edgerton and Kidder (31) and Sherbakoff (133) considered it non-pathogenic. The conflicting opinions are undoubtedly due to the variability of the fungus and the requirement of high soil temperatures for the development of seedling blight (76, 90, 91). Lately, Futrell et al. (38, 40) and others (27, 90, 91, 120) have reaffirmed the importance of F. moniliforme as a seedling blight pathogen.

Disease cycle Fusarium moniliforme overwinters in the soil or in plant debris as sclerotia, dormant perithecia,

or thickened hyphae (7, 101). Due to its poor competitive saprophytic ability, F. moniliforme accounts for two percent or less of the fungal flora of the soil (102). The fungus may remain viable in infected seed up to 10 years, although the percentage of infected kernels declines rapidly during the first 15 months (29, 69). The fungus may be localized between the pedicel and abscission layer (143), or underneath the pericarp (62), or the mycelium may have ramified throughout the endosperm and embryo (9, 83). Futrell (38) cites an unpublished thesis by Naqvi who found that F. moniliforme existed in a mycoplasmic stage within the seed.

Conidia of F. moniliforme may be wind-blown, water-splashed, or disseminated by insects (65). There is circumstantial evidence the fungus can be carried 300-400 km by strong air currents (105). Spore production generally peaks in late summer (Aug.-Sept. in the U.S.) when F. moniliforme represents up to 30% of the fungal flora isolated in air samplers over corn fields. Propagule density ranged from 2.5-160 per square centimeter on leaf surfaces, and from 5-50 x 10^4/ml of water in the leaf sheath (105). Various insects, such as picnic beetles (Glischrochilus quadrisignatus Say), European corn borers (Ostrinia nubilalis Hubner), sugarcane borers (Diatraea saccharalis Fabricus), and corn earworms (Heliothis zea Boddie), serve as vectors for the fungus, and create wounds suitable as infection sites

for wind-blown inoculum (66, 108, 138, 159).

Most workers (9, 62, 65, 66, 74, 81, 120, 151) feel that wind-borne conidia falling on the silks are the primary inoculum. The fungus grows along the silks, enters the kernels, and from there spreads to the pedicels, vascular cylinder, and finally the shank. In contrast to Diplodia ear rot, infections were rarely noted starting at the butt end of the ear (57, 62). Earliest infections were noted almost immediately following silking when the kernels were in the milk stage (grain moisture ca. 75%) (62, 65, 74, 120). The percentage of infection increased progressively until the kernels reached the mature stage (moisture ca. 28%) (67).

An alternate systemic route of infection has been suggested by Foley (37). Foley found F. moniliforme was present to some degree in all tissues of the corn plant, without any apparent symptoms at the time of collection. If this infection resulted from infected seed, two mechanisms may explain the lack of symptoms: first, "rapid elongation of the stem by activity of the intercalary meristem may result in temporary freedom from infection of internodal tissue" (37), and/or, the concentration of antifungal compounds (i.e. DIMBOA) in the young plant may arrest growth of F. moniliforme until the concentration of the compound(s) diminishes (157). Koehler (62) found that in some cases Diplodia and Cephalosporium ear rot resulted from systemic

stalk infection, but he never observed systemic infection with F. moniliforme. Young and Kucharek (163) found F. moniliforme was present in stalks prior to flowering and increased progressively until physiological maturity when 80% of the stalks were invaded. No correlation was found between seed infection and stalk infection, or between stalk infection and ear rot (69, 104, 106).

Spread among the kernels on an ear may be by fungal penetration through the intact pericarp of adjacent kernels, via tip caps, or through pericarp breaks or injuries caused by insects, birds, or silk cuts (62, 138, 156). As the kernels reach maturity, the pericarp offers some resistance to infection and further spread is limited to penetration via the tip cap (9). Johann (57) felt that the presence of suberin in considerable quantity in the pericarp or tip cap might be an important factor in resistance to fungal penetration. The black layer, formed at physiological maturity (81), is thought to present an impervious barrier to fungal penetration (57). Johann (57) hypothesized that several factors may influence the resistance of maize to Diplodia ear rot: (i) "the speed with which the hilar layer is closed, (ii) the thickness and compactness of the closing layer, and (iii) the completeness of the union of the closing layer with the ends of the suberinized membrane of the testa." No explanations have been postulated to account for

the random nature of infected kernels.

Epidemiology

Conidium production by F. moniliforme and ear rot development are greatly influenced by weather conditions during the growing season. In general, spore production is favored by dry spells followed by hot, rainy weather (15). Fusarium ear rot development in the United States is most pronounced in years with a dry July and high rainfall during August, September, and October (65). In years when harvesting is delayed, the amount of rainfall in November is also of considerable significance.

It has been observed that ear rots are particularly severe when maize is grown in soils high in organic matter (65). Fusarium ear rot is also more severe when phosphorus is low, or the nitrogen:phosphorus ratio is high (15, 50). Ooka and Kommedahl (104) reported less kernel infection by F. moniliforme in maize receiving supplemental nitrogen (600 kg/ha) compared to maize in unfertilized soil. Demopulos-Rodriguez (26) found no difference in ear rot severity (caused by all fungi) in opaque-2 maize grown with or without nitrogen fertilization (112 kg/ha). Grain protein and lysine concentrations were increased by nitrogen fertilization (26), but vegetative growth of F. moniliforme was similar on cornmeal obtained from maize grown on nitrogen-amended or

unamended soil (104). Sporulation of F. moniliforme, however, was enhanced on cornmeal from maize grown in nitrogen-amended soil.

The effect of previous cropping may have some bearing on Fusarium ear rot severity. Burrill and Barrett (15) stated that "....as a rule ear rot is more prevalent and destructuve in fields planted successively to corn...." Moore (92) found less Fusarium ear rot when maize followed alfalfa and postulated that lowered soil pH may have diminished the indigenous soil population of F. moniliforme. Koehler (65), however, reported no difference in Fusarium ear rot with various crop rotations, involving combinations of maize, wheat, oats, and soybeans.

The time of planting has little consistent effect on Fusarium ear rot development. In a six-year study, Koehler (65) observed more ear rot with late plantings than with early planted maize in three of the years, while no differences were detected in the other years. Time of harvesting, however, had a significant effect on ear rot development. Ear rot severity, caused by all fungi, increased in proportion to the time the corn was allowed to remain in the field past physiological maturity. As grain moisture decreased below 23%, growth of F. moniliforme was arrested, but ear rots caused by other fungi (Aspergillus and Penicillium spp.) continued (49, 61, 66). Some isolates of F. moniliforme will

grow at moisture levels as low as 18%.

The antagonistic interaction of various microorganisms, including Chaetomium globosum Kunze ex Fr., Bacillus subtilis (Cohn) Prazmowski, and Azotobacter sp., towards F. moniliforme has been noted by several workers (70, 86). In view of the lack of correlation between seed infection and ear rot (69), application of antagonistic microorganisms to maize seeds may have little effect on subsequent ear rot severity.

Various treatments have been tested in an effort to kill seed-borne F. moniliforme. Hot water treatment (53-60 C for 10 minutes) was reported by Niel and Brien (100) to kill the fungus, but there are many reports where this treatment was ineffective (74, 115, 151). Most fungicides commonly used as seed treatments are ineffective against internally-borne F. moniliforme (9, 133, 136, 151). Formalin, copper salts, Bordeaux mixture, thiram, captan, carboxin, and oxycarboxin all failed to control seed-borne F. moniliforme. Mercuric salts did kill the fungus, but the concentrations required were often phytotoxic (9, 74, 151). Mercurial compounds are not registered currently for use on maize seed. Benomyl and thiabendazole however, not only arrested the growth of F. moniliforme, but also were detected in 10-day-old seedlings in concentrattions sufficient to inhibit the fungus (39).

Ear rot resistance

No known maize hybrids or inbreds are immune to Fusarium ear rot, and many of the genotypes rated as highly resistant are not currently employed in maize breeding programs (6, 52, 63, 65, 139). Among inbreds presently in use, some do exhibit better combining ability for resistance to Fusarium ear rot than others. Unfortunately, resistance to a specific ear rot is largely independent of resistance to other ear rots, and resistance to a given ear rot is not correlated to seedling blight or stalk rot resistance (51, 65). Koehler (65) stated in 1959 "... not much advance in genetic control of ear rots has yet been made..." and the improvements gained so far have resulted from the use of erect, uniform maturing hybrids rather than open-pollinated varieties.

Traits influencing ear rot incidence Various plant characters have been shown to influence ear rot severity. Ear rot is more prevalent in ears poorly covered by husks than with ears with tight, complete husk coverage (62, 65). Maize hybrids with husks that open early are preferred by farmers because this allows the ears to dry faster. Koehler (62, 65) found that erect ears were more subject to seed infection than pendant ears, but this trait was judged less important than husk coverage. To aid in selecting disease resistant plants, Holbert et al. (50) used the criteria of complete husk coverage, a bright, clean shank, and kernels

with bright, rich luster. Large, well-developed kernels with a smooth indentation and a vitreous, rather than starchy endosperm were also considered desirable characteristics (50, 144, 145) Starchy kernels, irrespective of kernel infection, produced plants with less vigor, lower grain yields, and more barren stalks than kernels with vitreous endosperm. Ullstrup (148) observed that opaque-2 hybrids appeared to be more susceptible to ear rots than normal maize hybrids, with F. moniliforme and Penicillium sp. the principal fungi. This has been substantiated by other workers (76, 107, 108, 137) who have shown that opaque-2 maize may have two to four times more ear rot than normal maize. Ooka and Kommedahl (104), however, found no appreciable differences in ear rot between opaque-2 and normal maize, but as Loesch et al. (76) point out, the effect of the opaque-2 gene on ear rot susceptibility is influenced by the genetic background in which the gene is incorporated. Warmke and Schenck (154) found that maize with Texas male-sterile cytoplasm had twice as much seed infection with F. moniliforme, while others (39, 104) found no appreciable differences between normal and male-sterile cytoplasm.

Inoculation techniques In evaluating maize for ear rot resistance, one cannot rely on natural infection due to the yearly variation in both severity of rot and the fungi present. Numerous techniques of inoculating maize to produce

ear rots have been employed, including silk, tip-of-ear, husk, shank, leaf axil, and stalk inoculations (65). Stalk, shank, and leaf axil inoculation were all unsuccessful. Silk and tip-of-ear spray, and husk injection with spore suspensions were found by Koehler (65) to be the best methods. Conidial suspensions are usually obtained from cultures growing on potato dextrose agar, on sterilized oats, or on synthetic media (6, 65, 148). Boling et al. (6) found that ear inoculations made by shooting a spore-covered BB pellet into the ear were more successful and less time consuming than the silk or tip-of-ear methods suggested by Koehler (65). Ooka and Kommedahl (104) found the toothpick method of Young (162) revealed differences in susceptibility better than the silk spray method. All workers found that inoculations made within 10-20 days after pollination were most effective; once the kernel reached the dent stage, inoculations were generally unsuccessful.

Evaluation methods Various methods for evaluating ear rot include whole ear rating, sorting of kernels, germination tests, and plating of kernels on agar media, on rolled paper towels (ragdoll), or on paper blotters. Most workers that examine whole ears employ a 1-5 rating scale, either subjectively rating ears on the degree of rot (26, 107), or scoring on an estimate of the percent of rotted kernels (6, 139). Hoppe and Holbert (53) and Koehler (65)

felt kernel sorting was a more accurate method and less subject to bias than whole ear ratings, although vastly more time consuming. A representative 200 g sample, obtained with a Boerner divider, was judged the optimum sample size.

With both whole ear and kernel rating, no provision is included to assure that the rotted kernels are actually infected with a specific pathogen, other than the characteristic symptoms of the rotted kernels. This, coupled with the fact that many apparently healthy kernels are actually infected, has prompted some researchers (99, 104, 134) to use kernel infection as a basis for evaluation. The germination test, as used by the seed industry, has also been used to detect kernel infection (28, 154). The kernels are not routinely surface-sterilized, however, which may lead to elevated estimates of infected seed. If data are taken on the number of abnormal seedlings plus the number of dead seeds, this method would provide a good index of the effect of the fungi on seed performance. Germination tests performed in sand or on rolled paper towels may suffer from the problem of cross-contamination.

Singh et al. (134) found the deep-freezing blotter technique superior to the ragdoll method used by many workers (28, 154) in seed germination tests. Chlorine pretreatment reduced the recovery of F. moniliforme by eliminating surface-borne conidia, but was necessary to reduce

saprophytic contaminants (134). Ikenberry (56) found that soaking seed in aerated water for 24-48 hours increased the recovery of F. moniliforme, but unless the seed were soaked individually this may lead to contamination of healthy seed. Neergaard (99) recommends extended incubation up to 20 days to reveal deep seated infections, but at 28 C, incubations of more than four days were of little benefit (134). Incubation time was also reduced by using halved rather than whole kernels (69). Variations in light, including total darkness, diurnal light-dark cycles, or near ultra-violet light (320-400 nm) have little effect on the morphology, sporulation, or percentage recovery of F. moniliforme (134). Various selective media have also been developed to facilitate the isolation and identification of F. moniliforme (68, 130). Regardless of the technique used, F. moniliforme is easily distinguished from other species of Fusarium by the characteristic microconidia borne in chains, and the scarcity of macroconidia (7, 98). F. moniliforme is sometimes confused with Cephalosporium acremonium, from which it can be separated by the fact that C. acremonium grows slower and bears conidia only in false heads (98, 99).

Opaque-2 Maize

In terms of world production, maize is the second most important cereal grain. Although most is consumed by livestock, maize constitutes from 40-50% of the human diet in the developing countries of the world; in some areas of Latin America maize constitutes nearly 75% of the human diet (10).

The protein in maize is usually 9-11% of the grain weight (16). This would be adequate protein for human nutrition, but due to the low concentration of lysine and tryptophan, and excessive amounts of leucine, the human body utilizes about half of the available protein. If the lysine content could be raised from 2% found in normal maize to roughly 4%, all of the protein in maize could be utilized (10, 16).

In 1964, Mertz et al. (86) discovered that the opaque-2 gene in the recessive homozygous condition drastically altered the amino acid composition of maize by increasing the concentration of lysine and tryptophan. This increase was due to a reduction in the lysine poor zein fraction and an increase in the glutelin, globulin, and albumin protein fractions (86). In the decade since Mertz's discovery, plant scientists have rapidly incorporated the opaque-2 gene into many maize inbreds, varieties, and populations in an attempt

to improve the nutritional quality of maize for humans and monogastric animals (10).

Differences between normal and opaque-2 maize

Opaque-2 maize differs from normal maize in other attributes, many which are agronomically detrimental. These differences include reduced grain yield, a soft, chalky endosperm, and a greater susceptibility to insects and ear rotting fungi (1, 108, 109). Opaque-2 maize generally yields 10-15% less than normal maize (71, 141), primarily because of lower kernel weight and lower kernel density of opaque-2 maize (1, 2, 71, 109). Opaque-2 kernels contain less endosperm and have larger embryos (2, 43) and more pericarp tissue than normal maize (43, 46). Opaque-2 maize also dries slower than normal maize which results in higher moisture at harvest (71); Sperling (140), however, found no differences in moisture at harvest of normal and opaque-2 maize grown in the tropics. Dry matter accumulation was slower and ceased sooner in opaque-2 maize than normal maize (80, 97), but there was no difference in the time of black layer formation, indicative of physiological maturity (25, 80). Due to lower kernel density and higher moisture, opaque-2 kernels are softer than normals (77) and exhibit increased kernel breakage (71, 109). These characteristics are related to the smaller starch granule size (12) and the loose packing of

starch granules in the endosperm (123). Opaque-2 maize kernels contain higher concentrations of P, K, Mg, Fe, and Zn than normal kernels (30, 43). Aside from kernel traits, normal and opaque-2 plants differ little. Salamini (126) observed no differences between normal and opaque-2 maize for traits like ear height, ear length and diameter, date to pollen shed, leaf length, and leaf width.

A concern of plant scientists has been the performance of opaque-2 maize with respect to environmental stresses, insects, and disease-inciting organisms. Nass and Crane (95) reported that opaque-2 maize performed slightly poorer than normal maize in terms of percent germination, seedling height, and growth rate, but none of the differences was statistically significant. Gupta and Kovacs (47) tested six opaque-2 lines, plus all possible single, double, and three-way crosses, for percent germination, rate of emergence, and cold tolerance. Overall, opaque-2 genotypes were 30% lower in germination and rate of emergence, and were 35% inferior in cold tolerance than their normal counterparts. Opaque-2 three-way crosses, however, exhibited germination and cold tolerance characteristics superior to those of normal three-way crosses. Fungicide seed treatment improved the cold tolerance of opaque-2 seed, but not to the levels of normal maize seed. They postulated that the thicker pericarp and higher moisture of the opaque-2 kernels may have accounted

for the delayed germination and sensitivity to low temperatures.

Susceptibility to insects and fungal pathogens

Ear damage caused by corn earworms, sugarcane borers, and spotted sorghum borers (Chilo zonellus Swinhoe), and infestation of stored grain with maize weevils (Sitophilus zeamais L.) and the Angumois grain moth (Sitotroga cerealella Olivier) is usually more severe in opaque-2 maize than in normal maize (107, 108). The range of differences between opaque-2 and normal maize suggests that susceptibility to insect damage varies with the genetic background. No differences were noted in foliar damage caused by sugarcane borers or fall armyworms (Spodoptera frugiperda J. E. Smith) (108).

Ullstrup (149) in 1971 noted that opaque-2 hybrids appeared more susceptible to ear rot than normal maize hybrids, with F. moniliforme and Penicillium sp. the predominant fungi. This has been verified by several workers (76, 107, 108, 137) with various ear rotting pathogens, in both temperate and tropical environments. Ooka and Kommedahl (104) felt there was no difference in susceptibility between opaque-2 and normal maize, but five of the nine hybrids examined had more infected kernels in the opaque-2 version. When various inbreds, single or three-way crosses were examined under natural or artificial infection, the opaque-2 versions

were consistently more susceptible. Loesch et al. (76) examined the reaction of 20 inbred lines to ear rot caused by F. moniliforme, Gibberella zeae, Diplodia zeae, and Nigrospora zeae. On the average, the opaque-2 versions had disease ratings two to four times greater than the normal lines, with D. zeae causing the greatest increase in ear rot with opaque-2 germplasm. Lines rated by Loesch et al. (73) as resistant to one pathogen were not necessarily resistant to the other pathogens, as had been found earlier by Koehler (65) A few of the inbred lines (B57, C123, Oh7A, and Oh51A) were fairly resistant to all fungi tested, with the opaque-2 versions only slightly more susceptible than the normal maize inbred lines.

In contrast to ear rot damage, opaque-2 maize does not appear to be more susceptible to foliar pathogens (108) With regard to storage molds, Ortega (108) found little difference in the growth rate of Penicillium sp. or Aspergillus sp. on opaque-2 or normal grain. Lillihoj and Zuber (73) found no significant difference in aflatoxin B-1 levels between opaque-2 and normal maize. Nagarajan and Bhat (94) found that opaque-2 maize, in contrast to normal maize, contained higher concentrations of a protein factor which inhibited the production of aflatoxin.

Traits associated with disease incidence

Some of the kernel characters which differ appreciably between normal and opaque-2 maize have been shown to influence ear rot severity. Demopulos-Rodriguez (26) found ear rot scores were positively correlated with kernel fracture or breakage (.51), and negatively correlated with protein content (-.25), percent moisture at harvest (-.21), and test weight (-.30). Sadehdel-Moghaddam (124), utilizing the BSBB opaque-2 synthetic, found similar relationships between ear rot and kernel traits. Protein (.15), lysine (.18), and silking date (.45) were positively correlated with ear rot, while negative correlations were obtained between kernel hardness (-.18), moisture at harvest (-.23), kernel density (-.27), and ear rot scores. Ooka and Kommedahl (104) compared the growth of F. moniliforme on cornmeal agar made with opaque-2 and normal grain and found no difference in vegetative growth rate; sporulation, however, was appreciably greater on the opaque-2 cornmeal medium. This suggests that nutritional differences per se do not explain the greater susceptibility of opaque-2 maize. With regard to Diplodia ear rot, Johann (57) felt that variations in resistance may be due to the "the completeness of the union of the closing layer with the ends of the suberized membrane of the testa." Pugh et al. (118) concluded that resistance in wheat kernels to G. saubinetii was proportional to the thickness of the

testa or pericarp. Brown (13) observed that both the thickness and composition of plant cell walls determined the ability of the host plant to resist mechanical penetration by fungi. Opaque-2 kernels have been reported to have thicker pericarps than mormal kernels, but this is based on measurements of only one hybrid (46). In addition to the possible relationship with fungal penetration, pericarp thickness is negatively correlated with the speed of kernel drying (119). Genotypes with thicker pericarps may not attain moisture tensions that arrest fungal growth as soon as those with thinner pericarps and faster drying rates.

The concentration of cyclic hydroxamates (i.e. DIMBOA) in maize tissue has been shown to confer resistance to various fungal pathogens including F. moniliforme, G. zeae, and Helminthosporium turcicum Pass. (23, 149, 157). The concentration of DIMBOA is highest in root and stalk tissues of young plants and usually decreases by anthesis. Compared to the levels in stalks, the kernel endosperm contains but scant traces of cyclic hydroxamates (149). Thus, the levels of DIMBOA and related compounds in the kernels, and the possible differences between opaque-2 and normal maize kernels, probably has little bearing on the differential susceptibility of opaque-2 maize.

Alternatives to opaque-2 maize

Several alternatives to the use of opaque-2 maize for improved protein quality are currently being explored. These include the use of modified opaque-2 maize (49), the incorporation of other endosperm genes (88), and the development of high-lysine normal maize (42). Other avenues being explored are the supplementation of normal maize with soybean flour or other high protein sources (10), and the use of other high protein grains including barley and sorghum (93, 135). Various modifier genes have been found that decrease the opacity and increase the hardness of opaque-2 kernels (110). Lysine and tryptophan concentrations are generally lower than that of opaque-2 maize in the absence of modifier genes, although kernel weight and density approach that of normal maize (4).

At least seven other single genes are known which, in the recessive homozygous condition, increase the lysine concentration of maize endosperm (88). These include brittle-1 (bt-1) and brittle-2 (bt-2), floury-2 (fl-2), opaque-7 (o-7), Other genes, such as amylose extender (ae), dull (du), sugary-2 (su-2), and waxy (wx), affec shrunken (sh) -1, -2, and -4, and sugary-1 (su-1).t starch synthesis in the endosperm of maize and also increase the concentration of lysine and tryptophan over that found in normal maize (42). None of these endosperm mutants when used singly result in a lysine or tryptophan concentration which exceeds opaque-2.

However, in combination with opaque-2, all exceeded the single opaque-2 mutant, with bt-1/o-2, bt-2/o-2, sh-2/o-2, and su-2/o-2 producing the highest levels of lysine and tryptophan (42, 129). As with the single opaque-2 mutant, the increase in protein quality is usually accompanied by reduced grain yield, lower kernel weight and density, and a decrease in germination. The double mutant su-2/o-2 may show some promise in that it exceeds both opaque-2 and normal maize in kernel density and hardness (42).

Zuber (164) has shown that after three cycles of recurrent selection, lysine content of normal maize can be increased without specific endosperm mutants. Selection for lysine content alone resulted in increased protein. Paez et al. (111) found several inbred lines and U.S. Department of Agriculture introductions of maize that had high lysine values in the normal version.

Rather than attempt the creation of a single source of balanced protein, some researchers (10) are investigating the feasibility of supplementing normal maize to rectify the amino acid imbalance. Addition of lysine or tryptophan to a normal maize diet, or supplementation with fish protein concentrate, soybean flour, or egg protein all improved the protein efficiency ratio of normal maize. As diets in developing countries are also deficient in calories, vitamins, and minerals, it was felt that protein-supplemented

maize was superior to amino-acid supplemented normal or opaque-2 maize. Bressani (10) felt the ultimate goal was a lysine-in-protein value of 3% and an increase in protein content from 9% to 14%.

High-lysine strains of sorghum have been found with 3% lysine-in-protein and 16-18% protein (135). High-lysine barley strains have also been discovered (93). Again, the floury nature of the endosperm poses agronomic problems similar to those encountered with opaque-2 maize. One must also consider the acceptability of "different" cereal grains and the customs of the people when attempts are made to modify the diet of people in developing countries.

MATERIALS AND METHODS

Survey of Fungal Genera Responsible for Ear Rots

An opaque-2 version of the Iowa Synthetic BB, grown at the ISU Agronomy farm in 1975, was used in this study. Five selfed ears from each of 50 random S-2 families were examined for ear rot in mid-October. The ears were scored on a non-linear 1-5 scale as follows: 1 = 0-1% kernels infected, 2 = 1-10%, 3 = 10-25%, 4 = 25-50%, and 5 = 50-100%. This rating system was later changed to a linear scale, with increments proportional to the percent rotted area (1 = 0-1%, 10 = 1-10%, 25 = 10-25%, 50 = 25-50%, and 100 = 50-100%). Three ears from each family were harvested, air-dried, hand-shelled, and the seed from each family bulked. One-hundred-fifty kernels were processed per family for seed health determinations according to the method of Singh et al. (134). The grain was divided into three replications of 50 seed each. Kernels were rinsed in tap water for 10 minutes, surface-sterilized in 1% sodium hypochlorite for 5 minutes, and thoroughly rinsed in tap water. The seeds were arranged equidistant on sterile, blue-gray germination blotters in alcohol-rinsed polystyrene boxes (28 x 17 x 4.5 cm deep). In the first replication, the blotters were moistened with 75 ml sterile, distilled water. In subsequent replications, the

water was amended with 2,4-dichloro-6-nitroaniline (DCNA) at 500 mg/liter (a.i.), to inhibit the growth of Rhizopus sp. (103). The samples were incubated at room temperature (20 C) for 2 days, when most of the seeds had begun to germinate. The samples were placed in a freezer at -20 C overnight (12-18 hours), removed and allowed to incubate an additional 5-7 days under alternating 12 hour cycles of darkness and incandescent near-ultraviolet light. Fungal colonies were identified to genus with the aid of a dissecting microscope, or where necessary, a compound microscope. One-hundred random Fusarium colonies were transferred to water agar and potato sucrose agar plates for identification to species (7). The water agar plates were amended with propylene oxide-sterilized oat straw to enhance sporulation (7).

The germination of the 50 families was tested using standard procedures employed by the Iowa State University Seed Laboratory. Four replications of 50 seeds were arranged on moistened Kim-Pack ® (Kimberly-Clark, Neenah, WI), a high-density, absorbant cellulose pad. The seeds were incubated at 25 C under continuous fluorescent illumination. After 7 days, the number of germinated seeds and abnormal seedlings were recorded.

Two systemic fungicides, thiabendazole (2-[4-thiazoyl]-benzimidazole) and benomyl (methyl[1-(butylcarbamoyl)-2-benzimidazole carbamate], were evaluated for their effec-

tiveness in killing internally-borne F. moniliforme. In the first of two experiments, a bulked sample of opaque-2 seed was surface-sterilized in 1% sodium hypochlorite for 5 minutes and then was treated with either fungicide at 1250 mg a. i./kg seed. The fungicides were applied either as a water slurry or by acetone infusion (112) for 30 minutes. Four replications of 50 seed were incubated as described previously. In the second experiment, benomyl was applied either as a water slurry or by acetone infusion, at rates of 106, 312, and 1250 mg a. i./kg seed. An untreated control and an acetone control was included. Seed infection by F. moniliforme was recorded after 5 days incubation at room temperature.

Evaluation of Systemic Infection

The possibility that F. moniliforme ear rot may originate from systemic plant infection was investigated in two growth chamber experiments. Prior to the experiments, the interior of the growth chamber was cleansed with a detergent solution containing 1% sodium hypochlorite and thoroughly rinsed with tap water. All pots and soil used in the growth chamber were autoclaved (121 C for 45 minutes) to minimize the possibility of air-borne spores. Kernels from an infected seed lot (97% F. moniliforme) were surface-sterilized for 5 minutes in 1% sodium hypochlorite, rinsed,

and planted, one kernel per 5 cm pot. The plants were transferred to 25 cm clay pots after emergence, and the soil surface covered with aluminum foil, to exclude any sporulation originating from seed-borne organisms. Day-night temperatures of 29 and 24 C, respectively, and a 14 hour photoperiod (2200 lux) were maintained throughout the experiment. At anthesis, the ears were self- or sib-pollinated by hand to insure adequate kernel set. Ears were harvested at physiological maturity, dried at 38 C, and shelled. Fifty seed were examined for kernel infection using the deep-freezing blotter technique described previously. Stalks were surface-sterilized in 70% ethanol and 1 cm cross-sections excised from every node below the ear. Stalk samples were incubated in plastic boxes under the same conditions as the kernel samples. Stalk and kernel infection were recorded after 5 days incubation at 24 C. Conditions were identical in the second growth chamber experiment, except that a 2 cm deep layer of a paraffin-petroleum jelly mixture (40:60 w/w) was used to seal the soil surface. Surface-sterilized kernels and stalk sections were plated on Komada's (68) selective medium, instead of assaying infection with the deep-freezing blotter technique.

Inoculation Technique Experiment

The experiment was designed as split-plot with three replications. Treatment combinations (inbred line-inoculation method) were randomly assigned to whole plots consisting of two rows, and sampling dates comprised the sub-plots. The 10 inbreds used were A632o-2, B14o-2, B37o-2, B57o-2, H84o-2, Mo17o-2, N6o-2, N28o-2, Oh43o-2, and W64Ao-2. Inoculation treatments were (i) silk spray with F. moniliforme spores, (ii) silk spray check (distilled water only), (iii) ear puncture with a fungal encrusted toothpick, (iv) ear puncture check (sterile toothpick), and (v) noninoculated control.

The experiment was planted at the ISU Ross farm on May 12, 1976. Fifty seed were planted per 5.3 m double-row plot, with rows spaced 76 cm apart. The field was isolated from the nearest known source of normal maize pollen by 0.8 km and was surrounded by a 12 row border of an opaque-2 hybrid. After thinning, the final stand was about 37,000 plants/hectare.

For silk spray inoculations, F. moniliforme was grown on 750 g of boiled, sterilized millet in 1 liter Erlenmeyer flasks for 2-3 weeks. The contents of a flask were agitated in water to dislodge spores and mycelium, strained through several layers of cheesecloth, and then the spore concentration adjusted to 5,000 spores/ml. The spore suspension was

sprayed onto the silks 10 days after 50% silk with a low pressure hand sprayer. Silk spray check consisted of a water spray on the silks.

For ear puncture inoculum production, round toothpicks were boiled or autoclaved four to six times in excess water to remove fungitoxic compounds. The toothpicks were arranged vertically in 250 ml beakers and covered one-third their length with potato broth. The beakers were covered with aluminum foil and autoclaved (121 C for 30 minutes). The contents of the beakers were seeded with F. moniliforme spores and were allowed to incubate at room temperature until needed. The inoculum used to seed the beakers was a composite of several isolates of F. moniliforme, obtained directly from infected maize seed and plated onto water agar to verify the absence of any other organisms. This prevented loss of virulence, often noted during prolonged culture of fungal pathogens on artificial media, and also circumvented the variability between isclates. Prior to inoculation, the fungal-encrusted toothpicks were removed from the beakers and allowed to air-dry. Ears were inoculated 10 days after 50% silk by inserting a single toothpick through the husk perpendicular to the ear axis and midway between the butt and ear tip. Ear puncture check consisted of inserting a boiled, sterile toothpick into the ear. Noninoculated control ears received no treatment.

Ears were harvested at 2, 4, 6, and 8 weeks after inoculation. At each harvest, five ears were randomly picked from a plot, husked, and rinsed in 1% sodium hypochlorite for 2 minutes. After rinsing in running tap water, the ears were individually placed in moisture chambers and allowed to incubate at room temperature for 3 days. The moisture chamber consisted of an alcohol-rinsed plastic cup (11 cm diameter and 14 cm high) covered with a transparent polyethylene bag. An autoclaved "stand", consisting of a 2 cm piece of plywood pierced with a 5 cm nail, was used to hold the ear upright without touching the sides of the cup. Ear rot was rated on a linear scale (1-100), as described in the previous experiment. Incubation of the ears in the moisture chambers allowed the fungus in infected, yet symptomless kernels, to be expressed by sporulation on the infected kernels. In addition, fungal spread (radius of fungal growth along kernel rows from inoculation site) was measured on ears receiving the ear puncture treatments.

Diallel Evaluation of Ear Rot Resistance

Seed of a 10-parent diallel, including reciprocal crosses, was obtained from Dr. P. J. Loesch, Jr., Department of Agronomy, and planted at the ISU Ross farm in 1976. The inbreds used as parents for this diallel were B37o-2, B45o-2, B57o-2, B65o-2, B66o-2, C123o-2, Mo17o-2, N6o-2, N28o-2, and N31o-2. The 90 opaque-2 hybrids were laid out in a

randomized complete block design, with four replications. Thirty-four seed were planted on May 11-12 in single row 5.3 m plots, with the rows spaced 76 cm apart. After thinning to 15 plants/row, the final stand was about 37,000 plants/hectare. The entire field was surrounded with a border of hybrid opaque-2 maize, and was 0.8 km distant from the nearest known source of normal maize pollen. The ears were inoculated by spraying the silks with a spore suspension of F. moniliforme about 10 days after the average 50% silk date. Spore production was identical to that described in the previous experiment.

At harvest, essentially no ear rot had developed. The ears were incubated in plastic pots as described in the previous experiment. The grain moisture was too low to allow sporulation of F. moniliforme from infected kernels, thus the ears were soaked in water overnight at 4 C. Following this rehydration, the ears were immersed in 1% sodium hypochlorite for 2 minutes, rinsed, and placed in plastic pots. Ear rot was scored after 3 days on the linear 1-100 scale. Statistical analysis of ear rot data followed the method of Griffing (45).

Design II Experiment

Seed production

The material utilized in the experiments that follow was generated by use of Design II mating system, in which a series of male lines were crossed onto a series of female lines to produce a set of half-sib and full-sib families. Four male lines and four female lines were used within each of six sets, thereby producing four half-sib families with a common male parent, four half-sib families with a common female parent, and sixteen full-sib families within each set.

The F-1 seed for the Design II experiment was produced at the ISU Hinds farm in 1976. The general nursery design for each of the six sets consisted of four blocks of opaque-2 females (one row of each female line per block), with a different opaque-2 male row between each block of females, for a total of 20 rows per set. In addition, 12 four-row blocks of unreleased S-4 opaque-2 lines were included to substitute for inbreds which failed to flower in synchrony with the intended male or female row. The inbred seed was provided by Dr. P. J. Loesch, Jr., Department of Agronomy, and Mr. E. H. Cox, Clyde Black and Sons, Inc., Ames, IA. Thirty seed were hand planted in 9.1 m rows, spaced 76 cm apart. The first half of all rows was planted on May 19 and the second half 13 days later. The plots were thinned to 30 plants/row for a population density of 43,000/hectare. The entire nursery was

surrounded by four rows of a purple aleurone synthetic to aid in determining contamination with foreign pollen. Ears were harvested at physiological maturity, starting on September 20. Any ear with normal or purple kernels was discarded. After drying for 7 days at 38 C, the ears were shelled, and the seed from each entry bulked. One set, in which the inbreds failed to "nick" for simultaneous silking and pollen shed, was sent to Hawaii and Florida nurseries in the winter of 1976 for seed production. Pollinations in Florida and Hawaii were graciously done by Dr. P. J. Loesch, Jr., Department of Agronomy, and Mr. E. DeMay, Pfizer Genetics, Inc., Olivia, MN. The opaque-2 inbreds used as parents in the Design II are listed in Table 1.

Seedling blight evaluation

Inoculum production White proso millet (Panicum miliaceum L.) seed was boiled in excess tap water for 30 minutes, drained, and placed in 1 liter Erlenmeyer flasks to approximately one-fifth of capacity. The flasks were capped with foil and autoclaved twice (121 C for 60 minutes) on separate days. The flasks were seeded with F. moniliforme spores and incubated for 5-7 days at 25 C with daily shaking. The inoculum used to seed the flasks was a composite of several isolates of F. moniliforme, obtained directly from infected maize seed and plated onto water agar to verify the

Table 1. Opaque-2 inbred lines used as parents in six sets sets of the Design II experiment.

	Females		Males	
Set 1	Oh43	RW153R	A619	B14A
	W32	W64A	A632	W37A
Set 2	Ch45	R168	B66	MS315
	Ch51A	751015[1]	C123	751039[1]
Set 3	E45	M14	N6	R802
	E46	R182	R181B	751327[1]
Set 4	E37	N28	K41	751331[1]
	Mo17	N31	Mo20W	751440[1]
Set 5	E57	RVa36	Oh7A	R803
	CI64	Va35	R75	751444[1]
Set 6	E65	RVa43	B59	751030[1]
	C103		R109B	751062[1]
				751336[1]

[1]Unreleased S-4 opaque-2 lines supplied by Dr. P. J. Loesch.

absence of any other organisms. The millet inoculum was stored at 4 C until needed. For use in the seedling blight experiments, the millet inoculum was ground in distilled water in a a blender and the final concentration of the suspension adjusted to 0.1 g millet inoculum/ml.

Growth conditions Maize seeds were surface-sterilized in 1% sodium hypochlorite for 1-2 minutes and rinsed in running tap water. Eight hundred g of washed whilte silica sand was placed in a plastic pot (11 cm diameter and 1 liter capacity). Ten seeds of a given hybrid were placed pedicel down in the pot and covered with an additional 200 g of sand (1.8 cm deep). One pot of each hybrid was seeded with 10 ml of the millet inoculum suspension described above. Control pots received 10 ml of distilled water. All pots were watered with 150 ml of Knop's solution and covered with a transparent plastic bag to retain moisture. The pots were maintained in a growth chamber at 26 C with a 14 hour photoperiod for 7 days. Knop's solution is composed of 0.102 g ferrous sulfate heptahydrate chelated with 0.075 g disodium EDTA, 0.285 g calcium chloride dihydrate, 0.285 g potassium chloride, 0.285 g monobasic potassium phosphate, 0.322 g ammonium nitrate, 0.427 g magnesium sulfate heptahydrate, 1.57 mg manganese chloride tetrahydrate, 1 mg boric acid, 0.1 mg zinc sulfate heptahydrate, 0.02 mg copper sulfate pentahydrate, and 0.06

mg ammonium molybdata per liter of distilled water.

After 7 days in the growth chamber, the seedlings were removed from the pots and rinsed in running tap water to remove the sand. The roots and shoot above the seminal node were excised, blotted dry, and the total fresh weight of the roots, and shoots from a single pot obtained. The number of dead (ungerminated) and killed (germinated, but with no measurable root), seed were also recorded.

Field evaluation

The F-1 seed of the Design II was planted in two locations near Ames in 1977, the Beef Nutrtion Isolation Plot (BN) and the Veterinary Medicine Isolation Plot (VM). The experimental design was a randomized complete block with four replications. Replications within sets were blocked together to insure adequate pollination. Both plots were adequately isolated from the nearest known sources of normal pollen (>0.8 km). Thirty seed/entry were planted in 4.6 m single-row plots, spaced 76 cm apart, on April 28 and May 10, at the BN and VM plots, respectively. All plots were thinned to 15 plants/row after stand counts were made, for a final population density of 43,000/hectare. Ten days after the average tassel date, all ears within a set were inoculated with toothpicks, prepared as described earlier.

Traits examined Data were recorded for stand, tassel date, percent moisture at harvest, grain yield, ear rot, kernel rot, kernel weight, kernel volume, specific gravity, kernel hardness, pericarp thickness (germinal, abgerminal, and average), pericarp entirety, percent protein, percent lysine, and percent lysine-in-protein. The procedures for trait measurements are detailed below.

Stand: The number of plants/row was recorded at the three-leaf stage, approximately 3 weeks after planting.

Tassel date: The date on which 50% of the plants within a row were shedding pollen was recorded, and this was converted to the number of days that the row exceeded the earliest tassel date.

Percent moisture: Two kernel rows were removed from five ears immediately after harvest, weighed to the nearest 0.1 g, and dried in a forced air oven at 103 C for 72 hours. Percent moisture was calculated on a wet weight basis:

(wet weight-dry weight) x 100/wet weight

Yield: The total shelled grain weight after drying for 7 days at 38 C was added to 1.1 times the weight of the grain used for moisture determination, and the total weight expressed in quintals/hectare.

Ear rot: Ten ears per row were rated for percent rotted area on the linear 1-100 scale described earlier.

Kernel rot: A random 200 g sample was hand sorted and the weight of the visibly rotted and infected kernels recorded to the nearest 0.1 g (52).

Kernel weight, kernel volume, and specific gravity: Three-hundred healthy undamaged kernels were weighed to the nearest 0.1 g, and their displacement was measured in 95% ethanol to the nearest cm^3. Specific gravity was calculated by dividing the 300 kernel weight by volume (g/cm^3).

Kernel hardness: Three 10 g samples of healthy, undamaged kernels per row were crushed in a shear press (77) and the average force (kg/m) necessary for crushing was calculated. The Stein Breakage Tester (79) used by Paez et al. (109) to determine kernel hardness was tried on replicated samples but the variability experienced was too great to delineate differences in hardness.

Pericarp entirety: A random 100-kernel sample was soaked in 0.1% fast green dye (95% ethanol) for 1 minute, dried and examined (64). Wherever a break in the pericarp occurred, the endosperm stained blue-green. The kernels were separated into sound or damaged categories. This method revealed pericarp damage, but it was not possible to determine the completeness of the pericarp over the tip cap, and was thus discontinued. The spectrophotometric method of Chowdry et al. (18, 19) was also judged unsuitable for the same reason.

Pericarp thickness: Ten uniform, healthy kernels were soaked overnight in 25% glycerin, the tip and crown excised, and the pericarp removed. After drying for 4 hours, three measurements were made on both the germinal and abgerminal sides with a jeweled micrometer (161) to the nearest 0.0001 inch. The mean thickness of the pericarp was converted to micrometers. Two microscopic methods for measuring pericarp thickness which utilized Sudan IV (161) or potassium iodide (30) stains and thin secticning, were tried but judged too time consuming for the scale of the experiment.

Percent protein, lysine, and lysine-in-protein: A 15 g sample of healthy, sound grain was sent to Dr. Y. Pommeranz, USDA-SEA, U.S. Grain Marketing Research Center, Manhattan, Kansas. Nitrogen concentrations were determined on an automatic nitrcgen analyzer, and the percent N multiplied by 6.25 to obtain percent protein. Lysine deteriminations were made on a Beckman Automatic Amino Acid Analyzer and reported as percent of kernel weight. Lysine-in-protein was calculated by dividing the lysine percentage by protein percentage.

Statistical analysis A generalized analysis of variance (ANOVA) with expected mean squares for a Design II is depicted in Table 2. Ear rot and kernel rot data were converted by logarithmic transformation (i.e. log[(ear rot x 10) + 1]) to correct the skewed distribution; all other traits were analyzed as unconverted data. All analyses (ANOVA, cor-

Table 2. Sources of variation and expected mean squares in the Design II experiment.

Source	df[1]	Expected mean squares
Sets (S)	(s-1)	
Reps/S	s(r-1)	
Entries/S	s(mf-1)	$\sigma^2 + r\tau_{fm} + rf\tau_m + rm\tau_f$
Males (M)/S	s(m-1)	$\sigma^2 + rf\tau_m$
Females (F)/S	s(f-1)	$\sigma^2 + rm\tau_f$
M x F/S	s(m-1)(f-1)	$\sigma^2 + r\tau_{fm}$
Error	s(r-1)(mf-1)	σ^2

[1]Where s, r, m, and f are the number of sets, replications, males and females, respectively.

relations, means) were performed using the 1976 version of the Statistical Analysis System program (SAS76), developed by North Carolina State University. General combining ability (GCA), defined as the performance of a genotype averaged across a series of crosses, was calculated by subtracting the overall set mean from a particular half-sib family mean.

RESULTS AND DISCUSSION

Survey of Fungal Genera Responsible for Ear Rots

During the harvest of 1975, a study was made of the fungi causing ear rots on opaque-2 maize in a breeding nursery at the ISU Agronomy farm. Four genera, Fusarium, Penicillium, Aspergillus, and Rhizopus, comprised the majority of the fungi isolated, with Fusarium the predominant genus (Table 3). F. moniliforme accounted for 88% of all Fusarium species isolated, based on a random sample of 100 Fusarium-infected kernels. F. moniliforme var. subglutinans comprised 3% and the remainder consisted of F. equiseti and F. graminearum. When Rhizopus was present in the sample, it spread quickly to other kernels and made quantitative analysis of R. stolonifer difficult and often masked the presence of slower growing fungi. In the second and third replications, DCNA was used in the imbibition water and it effectively inhibited the spreading growth of R. stolonifer (Table 3). DCNA apparently did not affect the recovery of other fungi with the possible exception of Chaetomium sp. and Alternaria sp.

Results of these isolations are in general agreement with Ullstrup's (149) observation that F. moniliforme and Penicillium sp. were the predominant fungi associated with

Table 3. Percent kernel infection by various fungi isolated from naturally-infected grain of 47 opaque-2 families in the presence and absence of DCNA.

	% Infection			
Genus[1]	Untreated	DCNA[2]	Mean	Range
Fusarium	64.1	64.9	64.7	25-99
Penicillium	22.8	17.4	19.2	1-55
Aspergillus	12.9	9.2	10.4	0-52
Rhizopus	6.3	1.0**	2.8	0-16
Chaetomium	0.85	0.13**	0.4	0-3
Cladosporium	0.21	0.15	0.2	0-3
Alternaria	0.17	0.00**	0.17	0-1
Helminthosporium	0.04	0.04	0.04	0-1
Other genera	0.17	0.15	0.16	0-1
Total[3]	89.6	83.2*	85.3	57-100

*,**Significantly different from untreated at the 5% and 1% levels of probability, in this and subsequent tables.

[1]See text for species enumeration.

[2]DCNA (500 mg/l) present in imbibition water to inhibit Rhizopus.

[3]Total is less than cumulative sum because a single kernel may be infected with more than one organism.

ear rots of opaque-2 maize. Other workers (8, 31, 32, 33, 50, 66, 81, 87). found F. moniliforme to be the principal fungus associated with ear rots of normal maize, with kernel infections occasionally reaching 70-80% in some seed lots. In the eastern U.S., D. zeae is isolated more frequently than F. moniliforme from diseased ears of normal maize (32, 81). In the Midwest, the prevalence of D. zeae is much lower than in previous years (personal communication, various commercial maize breeders). Noticeably absent from this survey were several fungi commonly isolated from rotted ears, including Diplodia zeae, Cephalosporium acremonium, and Nigrospora zeae. C. acremonium is considered rather slow growing and may have been overgrown by other fungi; D. zeae and N. oryzae both exhibit a fairly rapid growth rate (99). These fungi either were not present due to the dry weather during the growing season, or possibly the incubation and test conditions favored the growth of other fungi and the exclusion of D. zeae and N. oryzae. Hesseltine and Bothast (49) have shown a definite succession of fungal organsims on maize ears during the growing season. They isolated very low numbers of Diplodia sp. and in addition, encountered no Aspergillus sp.

The following fungal species were identified:

Fusarium moniliforme Sheld.
F. moniliforme var. subglutinans Wr. & Reink.
F. equiseti (Cda.) Sacc.
F. culmorum (W. G. Sm.) Sacc.
F. graminearum Schw.

Penicillium chrysogenum Thom
P. notatum Westling
P. oxalicum Currie & Thom

Aspergillus candidus Lk.
A. clavatus Desm.
A. flavus Lk. ex Fr.
A. fumigatus Fres.
A. glaucus Lk. ex Fr.
A. niger v. Tiegh.

Rhizopus stolonifer (Ehr. ex Fr.) Lind.

Chaetomium globosum Kze. ex Fr.

Cladosporium herbarum (Pers.) Link

Alternaria tenuis Nees ex Cda.

Helminthosporium carbonum Ullstrup

Miscellaneous species included:

Epicoccum nigrum Lk.

Doratomyces stemonites Cda.

Curvularia pallescens Boedijn

Gonatobotrys simplex Cda.

Pithomyces maydicus (Sacc.) Ellis

Scopulariopsis brumpti Bain

Trichoderma viride Pers. ex Fr.

Phoma zeicola Ell. & Ev.

Ear rot scores were most highly correlated with infection by *Fusarium* (Table 4), which indicated the rot observed in the field was primarily *Fusarium* ear rot. No significant correlations were found between infection (by a specific genus) and percent germination or percent abnormal

Table 4. Phenotypic correlations among fungal infection, ear rot, and germination traits.

	Log Ear Rot	% Germination (A)	% Abnormal Seedlings (B)	A-B
Log Ear Rot	-	-.12	-.03	-.10
% Infected	.40**	-.02	-.01	-.02
% Fusarium	.58**	-.24	.21	-.24
% Penicillium	.38**	-.27	-.26	.28
% Aspergillus	.24	-.27	-.21	.26

seedlings (Table 4). This lack of association has been noted by many workers and may be due to avirulence of the fungus (72), antagonism with other soil-borne microorganisms (20, 70), and/or the site of infection within the kernel (9). Mathur et al. (83) found a significant negative correlation between percent infection by F. moniliforme and percent germination of sorghum, but invariably F. moniliforme was localized within the embryo. Thus, "whole ear" rot ratings or kernel infection may not be sufficient criteria in themsleves to predict host responce (i.e. germination) to fungal infection.

The systemic fungicides, benomyl and thiabendazole, effectively inhibited growth of internally-borne F. moniliforme at rates as low as 100 mg a.i./kg seed (Table 5). Futrell (39) found that benomyl effectively killed internally-borne F. moniliforme in maize seed, but not Helminthosporium maydis race T. Other fungicides, including carboxin, thiram, zineb, and captan, were ineffective against F. moniliforme in Futrell's tests (39). Seed infusion of benomyl or thiabendazole with acetone did not enhance the inhibition of F. moniliforme over the standard slurry application method (Table 5). Papavizas and Lewis (112) found that fungicide seed treatment by acetone infusion significantly reduced disease severity of cotton planted in Rhizoctonia solani Kuehn or Thielaviopsis basicola (Berk. & Br.) Ferr. infested

Table 5. Percent recovery of F. moniliforme from naturally infected opaque-2 maize seed treated with benomyl or thiabendazole (TBZ) applied as a water slurry or by acetone infusion in two experiments.

Acetone present[2]	Concentration (mg a.i./kg seed)		% F. moniliforme isolation[1]	
	TBZ	Benomyl	Exp. I	Exp. II
-	0	0	25	25
+	0	0	12	13
-	1200	0	2	
+	1200	0	4	
-	0	1200	1	
+	0	1200	2	
-	0	100		4
+	0	100		7
-	0	300		2
+	0	300		4
-	0	1200		1
+	0	1200		3

[1]Four replications of 50 seeds examined per treatment. LSD (0.05) = 4.8 and 4.1 for experiments I and II, respectively.

[2]The symbols '+' and '-' denote fungicide application by acetone infusion or by water slurry, respectively.

soil. The use of benomyl as a maize seed treatment against F. moniliforme is probably not warranted unless a given seed lot is known to contain a high percentage of infected seed.

Evaluation of Systemic Infection

Kernels from a seed lot having a high percentage (97%) of F. moniliforme-infected kernels were grown to maturity in two growth chamber experiments to examine the possibility of systemic plant infection and subsequent ear infection. Efforts were made to preclude the possibility of seed- or soil-borne F. moniliforme from becoming air-borne. Seven of the 15 plants in the first experiment bore usable ears, while only five usable ears were obtained in the second experiment. Percent kernel infection of the ears from both experiments is found in Table 6. In the first experiment, all seven ears yielded F. moniliforme, with kernel infection ranging from 8-36%, while in the second experiment only three of the five ears were infected, with kernel infection ranging from 4-46%. Overall, kernel infection was lower in the second experiment, which was partially due to a decline in infection of the original seed lot from 97% to 30% during the 18 months in cold storage. Thus, under conditions which minimized air-borne spores from reaching the silks, F. moniliforme-infected kernels resulted in subsequant ear infection.

Table 6. Presence of F. moniliforme in stalks and kernels of opaque-2 maize plants grown from infected seed in two growth chamber experiments.

	Plant No.	Stalk infection of successive nodes (N) and internodes (I) below the ear node							Kernel Infection %
		7 N/I	6 N/I	5 N/I	4 N/I	3 N/I	2 N/I	1 N/I	
Exp. 1	1	-/-	-/-	+/-	+/-	+/+	-/-	-/+	8
	2	+/-	+/+	-/-	-/-	-/+	+/+	-/+	36
	3	-/-	-/-	-/+	-/-	-/-	-/-	-/-	22
	4	-/-	-/-	-/+	-/-	-/-	-/-	-/-	12
	5	+/-	+/-	+/-	+/-	-/-	-/-	-/-	28
	6	+/+	+/+	+/-	-/-	-/-	-/-	-/-	16
	7	-/-	-/-	-/-	-/-	-/-	-/-	-/-	26
Exp. 2	1	+/-	+/-	+/-	+/-	-/-	-/-	-/-	42
	2	+/-	+/-	+/+	-/-	-/-	-/-	-/-	6
	3	+/-	+/+	+/-	+/-	+/-	+/-	-/-	4
	4	+/-	+/-	+/-	+/-	+/-	-/-	-/-	0
	5	+/-	-/-	-/-	-/-	-/-	-/-	-/-	0

[1]The symbols '+' and '-' denote that F. moniliforme was present, or not observed, respectively.

Stalk infection with F. moniliforme was localized primarily in nodal tissue; 60% of the stalk sections yielding F. moniliforme were infected only in the node (Table 6). Foley (37) isolated F. moniliforme from the nodes two to four times more frequently than from internodal tissue. He postulated that "rapid elongation of the stem ... may result in temporary freedom from infection of the internodal tissue." Stalk infection in this experiment, however, rarely reached the ear-bearing node (Table 6). This may have been due to a deficiency of the isolation technique and/or due to the presence of air-borne spores which infected the ears via the silks. Koehler (62) observed in some instances that Diplodia and Cephalosporium ear rot resulted from systemic stalk infection, but never observed systemic infection with F. moniliforme. Ooka and Kommedahl (104) found a positive but non-significant correlation between stalk infection and kernel infection by F. moniliforme. This suggests that either (i) kernel infection was not solely associated with infected stalks (i.e. systemic infection), or (ii) that different mechanisms may be involved in stalk and kernel resistance to F. moniliforme. Thus, a given genotype may be susceptible to stalk rot and yet resistant to ear rot. In agreement with this, Hooker (51) found no significant correlations between stalk rot caused by D. zeae or G. zeae and ear rot incited by either of the two fungi. If ear rot can

result from systemic infection, the examination of seed lots for F. moniliforme infection and/or seed treatment with benomyl might be advisable. In addition, the use of stalk rot resistant genotypes may preclude the systemic route of ear infection.

An experiment utilizing gnotobiotic maize culture would help resolve whether or not seed- or soil-borne F. moniliforme can cause systemic infection and ear infection. This would probably entail (i) the use of pasteurized soil in which an indigenous population of saprophytic microorgansims would be present to keep F. moniliforme from proliferating, (ii) removal of the kernel soon after germination to prevent introduction of F. moniliforme into the soil, and (iii) periodic air sampling to substantiate aseptic conditions. Positive results would indicate that systemic infection may constitute an alternative route for ear infection. Negative results, however, may not necessarily invalidate systemic infection, as different mechanisms may be functioning for resistance to stalk rot and ear rot.

Inoculation Technique Experiment

An experiment was conducted during 1976 to evaluate the effectiveness of two inoculation methods and to determine the optimum time for evaluating ear rot severity. The two methods involved either spraying the silks with a spore sus-

pension or puncturing the ear with a fungal-encrusted toothpick which was left implanted in the ear.

The ear rot data, based upon a visual estimate of the percentage of ear affected, were highly skewed to the lower end of the rating scale. Transformation of the data to the natural logarithm of the row means effectively corrected the skewness of the data distribution towards a more normal distribution. Significant differences in ear rot scores were found among the inbred lines tested, the methods of inoculation, and the dates of rating, and due to the various interactions (Table 7).

The table of date-method means (Table 8), averaged over the nine inbreds, illustrates the comparative effectiveness of the two inoculation methods and the linear increase in ear rot severity with time. Both the ear puncture and silk spray inoculations produced more ear rot than their respective checks or the control. The ear puncture method consistently produced higher ear rot scores compared to the silk spray inoculation method at every sampling date. The ear puncture method was thus clearly superior to the silk spray method. The ear puncture check, which consisted of implanting a sterile toothpick in the side of an ear, was as effective as the silk spray with inoculum (Table 8).

Other researchers working with Fusarium ear rot have noted the inadequacies of the silk spray method. Loesch et

Table 7. Analysis of variance for ear rot (logarithmic transformation) in the inoculation technique experiment.

Source	df	M.S.
Rep	2	2.5
Method (M)	4	17.3*
Line	8	6.9**
Line x M	32	1.5**
Error a	88	0.5
Date	3	35.6**
Date x M	12	1.0**
Date x Line	24	5.2**
Date x Line x M	96	0.6
Error b	270	0.5

Table 8. Date, method, and date-method means for log ear rot and untransformed ear rot (in parenthesis) from the inoculation technique experiment.

Date	Ear Puncture	Ear Puncture Check	Silk Spray	Silk Spray Check	Control	Date Mean
2	4.4 (8)	4.2 (7)	4.1 (6)	3.8 (4)	3.5 (3)	4.0 (5)
4	5.3 (19)	4.5 (9)	4.4 (8)	4.3 (7)	4.3 (7)	4.6 (10)
6	5.8 (32)	5.1 (16)	5.3 (19)	5.0 (15)	5.0 (15)	5.2 (18)
8	5.4 (22)	4.4 (8)	4.4 (8)	4.0 (5)	4.0 (5)	4.4 (8)
Method Mean	5.2 (18)	4.6 (10)	4.5 (9)	4.3 (7)	4.2 (7)	

LSD (0.05) = 0.17 for date means and 0.15 for method means.

al. (76) tested the ear rot reaction of 20 inbred lines, in normal and opaque-2 versions, using the silk spray method with F. moniliforme. Ear rot scores of the inoculated normal and opaque-2 lines averaged 25% lower than those of control lines receiving no inoculation. Only two of the inoculated lines had ear rot scores significantly higher than their controls. The failure of ear rot scores to exceed those of control lines is partially a reflection of the extreme susceptibility of the genotypes tested and the ubiquitous nature of F. moniliforme. Ooka and Kommedahl (104) examined the resistance of 12 maize cultivars (inbred lines, and commercial single- and three-way crosses) to Fusarium ear rot. Silk spray inoculation produced ear rot scores averaging 50% lower than those of the controls, while the ear puncture method gave ear rot scores 200% higher than the non-inoculated controls. Both Loesch et al. (76) and Ooka and Kommedahl (104) used rating scales different from the one I employed, which makes comparisons difficult. The general shortcoming of the silk spray inoculation method, however, is well substantiated.

The higher ear rot ratings obtained with the ear puncture method are partially due to a circumvention of the effect of a tight husk. A tight husk, completely covering the tip of the ear would tend to reduce the amount of inoculum reaching the kernels, either under natural infection

or with the silk spray inoculation method. The ear puncture method is also less influenced by the rate of silk drying, which would be expected to influence the effectiveness of the silk spray method. Both of these drawbacks of the silk spray method may be partially overcome by either peeling back the husk and/or injecting a spore suspension with a syringe. This, however, would tend to be more time-consuming, and therefore not as efficient in a large research program.

It may be argued that the silk spray method more closely approximates natural infection, and the ear puncture method, by circumventing husk coverage and rate of silk drying, gives an inacccurate evaluation of ear rot resistance. In rebuttal, it should be mentioned that insects feeding on the ear serve as vectors of the fungus and create wounds, which circumvent the effects of husk coverage and silk drying.

Kernel infection in this experiment was apparent prior to placing the ears in plastic pot moisture chambers, as early as 2 weeks after inoculation. Ear rot severity was always greatest on ears receiving the ear puncture treatment. The highest ear rot scores were observed 6 weeks after inoculation. The decline in ear rot scores at 8 weeks (Table 8) probably was a reflection of kernel maturity (i.e. low moisture), which limited the sporulation of F. moniliforme from infected kernels when the ears were incubated in the moisture chambers. Theoretically, one could rate ear rot

severity prior to physiological maturity and thus harvest only the desirable genotypes at a considerable savings of time and labor.

Significance of the line-by-method interaction (Table 7) can be explained by referring to Table 9.

Not only did the ear puncture method produce higher ear rot scores than the silk spray method, but the relative ranking of the inbred lines changed with the two methods. For example, A632o-2 was not significantly different from Oh43o-2 when the inbreds were inoculated with the silk spray method. With the ear puncture method, however, A632o-2 is rated significantly more susceptible than Oh43o-2. Thus, comparisons of relative ear rot resistance between experiments may be misleading when different inoculation methods are employed.

Ear rot scores, measured either by subjectively estimating the percent rotted area, or by measuring the radius of the diseased area from the point of inoculation, were highly correlated (.90). Both rating methods were highly efficient for distinguishing resistant genotypes (Table 10) and the relative ranking of the lines did not differ significantly between the two rating methods (Table 11). Measurement of the diseased area (when using the ear puncture method) would be subject to less bias than subjectively estimating rot severity, and thus would be helpful when evaluations are made

Table 9. Relative rankings of opaque-2 inbreds based on ear rot ratings by either the ear puncture or silk spray inoculation method.

Ear puncture		Silk spray	
Inbred	Rating[1]	Inbred	Rating[1]
Oh43	4.18 a[2]	Oh43	3.62 a
N6	4.65 ab	A632	3.94 a
B37	8.6 bc	N6	4.56 b
H84	5.01 bcd	H84	4.59 b
W64A	5.26 bcde	N28	4.64 b
A632	5.53 def	W64A	4.77 b
B14	5.70 ef	Mo17	4.80 b
N28	5.83 ef	B37	4.83 b
Mo17	5.90 f	B14	5.09 b

[1] Averaged over four sampling dates and three replications. Ear rot rated on a linear 1-100 scale and corrected by logarithmic transformation.

[2] Ratings not followed by a common letter are significantly different at the 5% level of probability.

Table 10. Analysis of variance for ear rot (visual estimate of rotted area) and spread (radius of rotted area) from the inoculation technique experiment.

Source	df	Mean Squares	
		Ear Rot	Spread
Rep	2	.75	.03
Line (L)	8	4.67**	21.30**
Method (M)	1	4.41**	2.47**
L x M	8	.76	.31
Error a	34	.39	.20
Date	1	8.52**	10.88**
Date x L	8	3.47**	2.47**
Date x M	1	.04	.43
Date x L x M	8	.86*	.65*
Error b	36	.44	.29

Table 11. Ear rot scores of nine opaque-2 inbred lines rated by visually estimating percent rotted area or by measuring the radius of the rotted area.

Estimated		Measured	
Inbred	Score[1]	Inbred	Score[1]
Oh43	4.19 a[2]	Oh43	4.45 a[2]
N6	5.25 b	H84	5.26 b
H84	5.32 b	N6	5.38 b
B37	5.37 b	W64A	5.58 bc
W64A	5.57 bc	B37	5.88 bc
B14	5.78 bc	B14	5.70 bc
A632	5.80 bc	A632	5.77 bc
Mo17	6.36 c	Mo17	6.06 c
N28	6.39 c	N28	6.08 c

[1] Averaged over three replications and two sampling dates. Scores were corrected by logarithmic transformation.

[2] Ear rot scores not followed by a common letter are significantly different at the 5% level of probability.

by more than one person.

Diallel Evaluation of Ear Rot Resistance

Forty-five opaque-2 single-cross hybrids and their reciprocals, generated from a 10-parent diallel mating design, were evaluated for Fusarium ear rot resistance in 1976. Ears were inoculated about 10 days after the average 50% silking date with aconidial suspension of F. moniliforme using the silk spray method. Ears were evaluated for ear rot severity at harvest on a linear 1-100 scale and row means were transformed by taking the natural logarithm. The diallel mating design allows one to partition the effects due to the hybrids into general combining ability (GCA), specific combining ability (SCA), maternal, and reciprocal effects.

Ear rot development in this experiment was very slight, necessitating incubation of the ears in plastic pot moisture chambers. The failure of disease development was due in part to the use of the silk spray inoculation method, which was a poor inoculation method in 1976 (see previous section), and also to the extremely dry weather in July, August, and September. Koehler (62) has shown that ear rot severity is highly correlated with rainfall during the latter part of the growing season, and the drought during 1976 undoubtedly curtailed ear rot development.

Table 12. Analysis of variance for ear rot in the ten parent opaque-2 diallel, with entry mean squares partitioned into general combining ability (GCA), specific combining ability (SCA), maternal and reciprocal effects.

Source	df	Mean squares
Rep	3	9.46*
Entry	89	.83**
GCA	9	.78**
SCA	35	.17
Maternal	9	.06
Reciprocal	36	.14
Error	267	.4

As shown in the analysis of variance (Table 12), there were significant differences among hybrid entries and among the GCA effects, for the trait ear rot. The effects due to SCA, maternal, and reciprocal effects were all non-significant. Thus in the material examined additive genetic variation (GCA) was important, while non-additive genetic variation (SCA) was not. Maternal effects were not expected to be of significance as they are usually only of importance with F-1 kernel and seedling traits.

Ear rot ratings of the 90 entries and their maternal and paternal half-sib family means are found in Table 13. The inbred lines B45o-2 and B65o-2 exhibited the best GCA's for ear rot resistance while B57o-2 and C123o-2 had the poorest combining abilities for ear rot resistance. Unfortunately, earlier surveys of F. moniliforme resistance in maize did not include any of the inbreds used in this diallel (63, 139). In a more recent study, Loesch et al. (76) rated B57o-2 and C123o-2 as resistant and N6o-2 and N31o-2 as susceptible to F. moniliforme. The ear rot resistance of three of the inbreds (B57o-2, N6o-2, and N31o-2) compared well with their performance in hybrid combination in the diallel, while C123o-2 was much poorer in conferring resistance to its progeny in the diallel evaluation.

Table 13. Hybrid means, male and female means, and general combining ability (GCA) effects for log ear rot in a 10 parent opaque-2 diallel.

	Male										Female Means	GCA Effects
Female	B37	B45	B57	B65	B66	C123	N28	N31	N6	Mo17		
B37	-	3.12	4.11	4.00	4.34	4.55	4.18	4.55	4.67	3.25	4.09	-.09
B45	3.99	-	4.17	4.25	3.42	4.35	3.95	4.51	3.82	3.37	3.98	-.35
B57	4.46	4.19	-	4.11	4.56	4.36	4.87	4.67	4.49	4.33	4.45	.21
B65	4.04	4.00	3.95	-	4.52	4.00	3.33	3.33	3.93	4.91	4.00	-.27
B66	4.53	4.06	3.82	3.67	-	4.77	3.75	4.68	4.54	4.07	4.21	.00
C123	4.46	3.49	5.23	3.67	4.30	-	4.71	4.79	4.52	4.99	4.4	.26
N28	4.13	3.61	4.61	3.87	4.25	4.65	-	3.93	4.61	4.25	4.21	-.05
N31	4.47	4.40	4.80	4.01	4.12	5.09	4.47	-	4.34	3.65	4.37	.11
N6	3.81	3.93	4.68	4.04	4.55	4.35	4.86	4.61	-	4.48	4.37	.15
Mo17	4.26	3.69	4.82	4.07	4.68	4.92	3.69	4.13	5.03	-	4.37	.04
Male Means	4.24	3.83	4.47	3.97	4.30	4.56	4.20	4.36	4.44	4.14		

LSD (0.05) = 0.88 for hybrid means, 0.28 for male and female means, and 0.19 for GCA effects.

Design II Experiment

Ninety-five opaque-2 single-cross hybrids, generated by crossing 48 inbreds in a Design II mating plan, were grown at two locations near Ames in 1977. The primary objective was the evaluation of Fusarium ear rot resistance, and secondarily, the evaluation of kernel and agronomic traits and their relationship with ear rot resistance. The opaque-2 inbred lines used as male and female parents in the experiment are listed in Table 1. Nine public lines (B45o-2, H26o-2, H84o-2, K55o-2, MS321o-2, MS334o-2, RCI21Eo-2, Wf9o-2, and 38-11o-2) were replaced by unreleased S-4 lines when the original lines failed to flower in synchrony with the intended male or female counterpart. Since fixed lines were employed in this experiment, and not random lines derived from some population, inferences cannot justifiably be extrapolated to other maize inbreds, hybrids, or populations. The 39 public inbred lines used in this study represent a fairly wide cross section of the germplasm currently utilized in commercial hybrid production. A relatively small number of inbreds, however, are used as parents in the production of hybrid maize seed in the U.S. For example, inbreds A632, Mo17, B37, and A619 accounted for 33% of the hybrid seed production in 1975 (165). No information is available concerning the usage of specific opaque-2 inbreds in hybrid seed production. In the U.S., opaque-2 maize ac-

counts for 1% or less of the total acreage planted to maize (14).

The Beef Isolation plot was abandoned due to drought and racoon damage, therefore only the data from the Veterinary Medicine plot were analyzed. This loss was unfortunate as the various interactions with location may have altered the significance of the single-order effects. The mean of all traits, including their ranges, least standard deviations, and coefficients of variation (CV), are shown in Table 14. All traits were analyzed as raw data with the exception of ear rot and kernel rot. The mean and range for untransformed ear rot data was 5.8% and 1.4-73%, respectively, and for untransformed kernel rot data, 9.5% and 1.1-52%. Logarithmic transformation effectively corrected the skewness of the data distribution for both traits. Protein quality traits (% protein, % lysine, and % lysine-in-protein) and kernel quality traits were comparable to those reported by others (1, 109, 111, 141). Most of the traits, with the exception of stand, ear rot, and yield, had relatively low CV's (under 10%), indicating relatively good precision in measuring the traits and low variation for these traits within the lines. Yield data should be viewed cautiously, since the drought seriously affected grain yield, and also because single-row plots in one location were used for yield determinations.

Table 14. Means, ranges, least standard deviations (LSD) and coefficients of variation (CV) for all traits in the Design II experiment.

Trait	Mean	Range	LSD (.05)	CV (%)
Stand	22.4	17.5-26.8	4.3	14.0
Tassel date (days)	9.0	1-14	1.1	8.4
Ear rot (log % area)	4.07	2.7-6.6	0.76	13.5
Kernel Rot (log % weight)	4.56	2.4-6.3	0.43	6.7
300 Kernel Weight (g)	72.6	48-93	3.8	3.8
300 Kernel Volume (cm^3)	81.	52-106	3.7	3.3
Specific Gravity (g/cm^3)	1.11	1.03-1.22	0.03	2.2
Moisture (%)	22.8	15.9-32.8	2.4	7.7
Kernel hardness (kg/m)	13.9	10.1-18.2	0.9	4.7
Pericarp thickness (um)				
Germinal	69.	42-95	6.1	6.4
Abgerminal	56.	35-75	4.6	6.0
Average	62.	39-80	4.9	5.6
Protein (%)	10.6	9.0-12.3	0.5	3.6
Lysine (%)	0.42	.35-.50	0.03	4.5
Lysine-in-protein (%)	3.9	3.5-4.3	0.2	4.4
Yield (q/ha)	44.3	16.2-65.8	11.7	19.1

Table 15. Analyses of variance for stand, tassel date (Tas), ear rot (Erot), kernel rot (Krot), kernel volume (Vol), kernel weight (Wt), specific gravity (SpGr), and kernel hardness (Hard) in the Design II experiment.

		Mean squares							
Source	df	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Hard
Set (S)	5	86**	471**	13.7**	8.3**	4075**	6765**	611**	160**
Rep/S	18	16	1*	0.3	0.3**	18**	28**	54**	2**
Entry/S	89	15**	10**	2.3**	1.2**	177**	229**	30**	7**
Male (M)/S	19	21**	23**	2.7**	2.1**	372**	522**	53**	16**
Female (F)/S	17	15	20**	4.0**	2.1**	425**	458**	64**	12**
M x F/S	53	13	2**	1.3**	0.7**	46**	51**	8*	2**
Error	267	10	0.6	0.3	0.1	7.7	7.0	1.2	0.4

Table 16. Analyses of variance for moisture (Moist), abgerminal pericarp thickness (PA), germinal pericarp thickness (PG), average pericarp thickness (PAV), % protein (Prot), % lysine (Lys), % lysine-in-protein (L/P), and yield in the Design II experiment.

		Mean squares							
Source	df	Moist	PA	PG	PAV	Prot	Lys	L/P	Yield
Set (S)	5	600**	2911**	1963**	2216**	5.43**	2.38**	1.38**	1359**
Rep/S	18	10**	54**	97**	68**	.25*	.10**	.09**	77**
Entry/S	89	25**	424**	194**	255**	1.30**	.22**	.09**	355**
Male (M)/S	19	50**	1014**	477**	617**	3.83**	.53**	.18**	810**
Female (F)/S	17	58**	861**	360**	511**	1.64**	.44**	.16**	348**
M x F/S	53	5*	74**	42**	45**	0.30**	.05*	.04	159**
Error	267	3.1	19.5	11.1	12.3	0.15	.04	.03	71.5

Analyses of variance for the field experiment are presented in Tables 15 and 16. Significant differences were found among the hybrid entries for all traits. When the mean squares associated with hybrid entries were partitioned, the effects due to males, females, and the male by female interaction were statistically significant for all traits. Mean squares associated with males (GCA), and with females (GCA) were always greater than those associated with the male by female interaction (SCA). This implies that additive genetic variation (GCA) was of more importance than non-additive genetic variation (SCA) in the inheritance of the traits examined.

Correlations among ear rot, kernel rot, % protein, % lysine, % lysine-in-protein, and all other traits are found in Table 17. Correlations with kernel rot were generally of greater magnitude than those with ear rot. It was felt that kernel rot measurements were more precise than the subjective estimation of ear rot scores. Significant negative correlations were found between kernel rot and stand, tassel date, kernel specific gravity, kernel hardness, percent moisture at harvest, and yield. This would indicate that late-maturing genotypes with hard, dense kernels were more resistant to F. moniliforme ear rot. This is in agreement with previous work (26, 124) where ear rot was negatively correlated with kernel moisture at harvest, kernel density, and kernel hardness or

Table 17. Phenotypic correlations among ear rot (Erot) kernel rot (Krot), protein (Prot), lysine (Lys) lysine-in-protein (L/P), stand, tassel date (Tas), kernel volume (Vol), kernel weight (Wt), kernel specific gravity (SpGr), kernel hardness (Hard), moisture at harvest (Moist), abgerminal pericarp thickness (PA), germinal pericarp thickness (PG), average pericarp thickness (PAV), and yield in the Design II experiment.

	Erot	Krot	Prot	Lys	L/P	Stand	Tas	Vol	Wt	SpGr	Hard	Moist	PA	PG	PAV	Yield
Erot		.81 **	.15	.27 **	.20	-.35 **	-.06	.11	.00	-.44 **	-.52 **	-.10	-.06	-.10	-.09	-.20
Krot	.81 **		.18	.40 **	.34 **	-.26 **	-.28 **	-.12	-.22 *	-.51 **	-.41 **	-.25 **	-.12	-.13	-.13	-.43 **
Prot	.15	.18		.69 **	-.21 *	-.12	-.15	-.07	-.11	-.21 *	.05	.02	.28 **	.13	.23 *	-.25 *
Lys	.27 **	.40 **	.69 **		.56 **	-.04	-.43 **	-.28 **	-.41 **	-.69 **	-.35 **	-.36 **	.03	-.12	-.04	-.43 **
L/P	.20	.34 **	-.21 *	.56 **		-.09	-.40 **	-.30 **	-.43 **	-.69 **	-.54 **	-.50 **	-.28 **	-.32 **	-.32 **	-.31 **

breakage. The negative correlation between stand and kernel rot or ear rot indicates that seedling performance in the field may be associated with ear rot resistance of the mature plant. Stand counts are a composite indication of emergence potential for a given genotype, plus the ability of that genotype to resist soil-borne pathogens.

The correlations between kernel rot and percent lysine or percent lysine-in-protein were positive and significant, while no significant correlation was found between kernel rot and percent protein (Table 17). In contrast, Demopulos-Rodriguez (26) found ear rot was correlated only with protein, and not with lysine, tryptophan, lysine-in-protein, or tryptophan-in-protein. Sadehdel-Moghaddam (124) found ear rot was correlated with both protein and lysine, both not with lysine-in-protein. This lack of agreement among researchers is partially due to the different genotypes examined and also to the fact that the other two researchers used a subjective estimation of ear rot severity, thus decreasing the precision of the correlations. Ooka and Kommedahl (104) could demonstrate no difference in the vegetative growth rate of F. moniliforme on cornmeal agar made with either normal or opaque-2 maize. Thus, protein quality differences per se among opaque-2 genotypes may not account for the differences in ear rot severity. Rather, the decreased kernel density and kernel hardness associated with genotypes having high

protein and lysine levels (Table 17) may allow the fungus to penetrate and proliferate in these kernels and thus cause greater ear rot damage.

Kernel rot was not significantly correlated with any of the pericarp measurements. Pericarp thickness at harvest probably had little influence on infection by F. moniliforme. Richardson (122) has shown that pericarp thickness in popcorn decreases due to stretching caused by enlargement of the endosperm. Once endosperm growth ceases, however, the pericarp increases in thickness due to lignification. Thus pericarp measurements of genotypes differing widely in maturity may not be comparable. In addition, no information is available concerning the relationship between pericarp thickness at maturity and at earlier stages of kernel development such as the milk or early dough stage when infection takes place.

Traits negatively correlated with ear rot severity were unfortunately also negatively correlated with all protein quality traits. This correlation does not infer a cause and effect relationship. Late-maturing varieties with dense, hard kernels (conferring ear rot resistance) were in general low in percent protein and lysine. Fortunately, a few of the lines in hybrid combination exhibited good ear rot resistance without appreciably compromising protein quality. The half-sib families of B45, B46, C103, and Oh51A (used as females)

Table 18. Maternal half-sib family means from the Design II experiment for ear rot (Erot), kernel rot (Krot), % protein (Prot), % lysine (Lys), % lysine-in-protein (L/P), yield, specific gravity (SpGr), kernel hardness (Hard) and % moisture (Moist).

Female	Erot	Krot	Prot	Lys	L/P	Yield	SpGr	Hard	Moist
W32	4.39	4.95	10.6	.44	4.18	29.3	1.06	12.2	16.8
W64A	5.11	5.21	11.5	.46	3.96	46.3	1.09	13.6	19.6
RW153R	5.06	5.32	11.5	.47	4.10	36.5	1.05	10.8	19.4
Oh43	4.53	5.03	10.8	.43	3.98	33.8	1.07	12.5	20.6
751015	3.72	4.51	10.0	.39	3.87	56.0	1.13	14.6	19.5
R168	3.71	4.68	10.4	.41	3.99	42.3	1.11	14.2	19.7
Oh45	3.70	4.76	10.5	.41	3.91	40.3	1.12	14.6	20.4
Oh51A	3.45	4.33	10.5	.42	4.03	47.5	1.12	15.2	20.3
R182	3.90	4.21	10.2	.40	3.97	46.2	1.11	14.3	20.8
M14	4.18	4.85	10.0	.41	4.08	41.2	1.11	13.8	21.5
B45	3.72	4.58	10.2	.43	4.16	39.0	1.09	13.6	24.0
B46	3.57	4.23	10.8	.46	4.28	42.2	1.08	13.6	21.3
LSD (0.05)	0.38	0.21	0.3	.01	0.12	5.9	0.01	0.5	1.2

Table 18 continued.

Female	Erot	Krot	Prot	Lys	L/P	Yield	SpGr	Hard	Moist
Mo17	4.20	4.32	10.5	.40	3.77	48.8	1.14	15.0	23.9
N28	3.34	3.91	10.5	.38	3.60	50.4	1.20	16.0	29.6
N31	3.95	4.28	10.6	.41	3.88	49.7	1.12	13.7	24.4
B37	2.99	3.60	10.4	.39	3.75	50.5	1.15	15.3	22.4
B57	4.74	5.07	10.4	.41	3.90	48.3	1.12	13.6	27.3
Va35	3.91	4.66	10.8	.40	3.71	45.0	1.16	15.1	26.6
RVa36	4.35	4.47	11.2	.41	3.69	40.5	1.11	14.1	26.5
CI64	4.75	4.69	10.4	.40	3.86	45.7	1.13	14.0	27.9
C103	3.48	4.34	10.6	.43	4.04	45.2	1.12	14.8	23.9
B65	5.21	5.24	10.9	.41	3.96	45.4	1.09	13.2	21.2
RVa43	3.69	3.85	10.1	.40	3.97	48.1	1.13	12.4	26.4
LSD (0.05)	0.38	0.21	0.3	.01	0.12	5.9	0.01	0.5	1.2

Table 19. Paternal half-sib family means from the Design II experiment for ear rot (Erot), kernel rot (Krot), % protein (Prot), % lysine (Lys), % lysine-in-protein (L/P), yield, specific gravity (SpGr), kernel hardness (Hard) and % moisture (Moist).

Male	Erot	Krot	Prot	Lys	L/P	Yield	SpGr	Hard	Moist
W37A	4.66	4.82	10.2	.41	3.99	41.6	1.10	12.4	19.0
A619	4.53	5.23	11.0	.44	4.05	32.3	1.06	11.8	18.9
A632	5.06	5.34	11.2	.47	4.15	38.0	1.06	12.1	18.5
B14A	4.84	5.13	11.9	.48	4.03	33.8	1.06	12.8	20.1
751039	3.57	4.27	10.5	.41	3.91	49.1	1.14	14.7	19.8
MS315	3.49	4.72	10.1	.42	4.17	38.1	1.09	13.5	16.7
C123	3.76	4.61	10.0	.38	3.85	55.2	1.14	15.6	21.8
B66	3.77	4.68	11.0	.42	3.86	43.8	1.11	14.9	21.6
N6	3.73	4.87	10.7	.46	4.15	34.6	1.11	15.2	24.8
R181B	4.29	4.26	9.9	.41	4.11	48.6	1.08	12.3	19.7
R802	4.17	4.78	10.3	.43	4.14	38.8	1.10	14.1	22.1
751327	3.18	3.96	10.3	.42	4.10	46.6	1.09	13.6	20.9
LSD (0.05)	0.38	0.21	0.9	.01	0.12	5.9	0.01	0.5	1.2

Table 19 continued.

Male	Erot	Krot	Prot	Lys	L/P	Yield	SpGr	Hard	Moist
751331	3.36	3.72	10.5	.40	3.76	53.8	1.14	14.7	22.5
K41	3.55	3.60	10.4	.39	3.71	52.0	1.16	14.5	26.8
751440	3.82	4.26	10.6	.41	3.82	55.7	1.14	15.1	25.2
Mo20W	3.76	4.53	10.5	.39	3.71	38.0	1.16	15.7	25.9
R803	4.29	4.58	10.7	.40	3.72	36.0	1.15	14.9	27.3
R75	4.27	4.72	11.5	.43	3.73	41.6	1.12	14.4	27.3
751444	4.06	4.46	10.9	.40	3.67	59.7	1.13	15.3	25.5
Oh7A	5.13	5.13	9.7	.39	4.04	42.3	1.10	12.2	28.1
751336	4.65	4.60	10.9	.42	3.91	53.6	1.10	13.0	26.3
751062	3.70	4.48	9.9	.40	4.03	49.5	1.12	13.3	22.0
751039	3.73	4.15	10.2	.42	4.15	47.0	1.10	12.4	25.2
B59	5.09	5.26	10.6	.42	3.97	43.1	1.09	13.0	21.6
R109B	3.47	3.90	10.5	.41	3.90	37.9	1.14	15.8	24.0
LSD (0.05)	0.38	0.21	0.9	.01	0.12	5.9	0.01	0.5	1.2

and B66, N6 and 751327 (used as males) all exhibit good ear rot resistance and have lysine values greater than 0.42% (Tables 18 and 19). The opaque-2 inbreds B37, N28, R109B, and 751331 also displayed good ear rot resistance in hybrid combination, but had less than optimum protein and lysine levels. Several widely used inbreds exhibited rather poor half-sib family means for ear rot resistance. These included B65, RW153R, and W64A (used as females) and A632, B59 and Oh7A (used as males).

The general combining ability (GCA) of all inbreds for pertinent traits are shown in Tables 20 and 21. The ranking of inbreds within a given set remained the same, whether the half-sib family mean or the GCA was used, since GCA is merely the deviation of a half-sib family mean from the overall set mean. When comparisons were made across sets, however, inbreds with similar half-sib family means did not necessarily have similar GCA's, due to the differences in set means. For example, B46o-2 and C103o-2 had similar half-sib family means for ear rot of 3.57 and 3.48, respectively, while their respective GCA's were -2.7 and -6.5. Some people argue that comparisons across sets are invalid since, in the Design II, there are no parents in common between sets. Others feel that comparisons of GCA's across sets are valid, but only if sets are not significantly different. As shown previously in Tables 15 and 16, differences among sets were statistically

Table 20. General combining abilities of the opaque-2 inbred lines used as females in the Design II experiment for ear rot (Erot), kernel rot (Krot), % protein (Prot), % lysine (Lys), % lysine-in-protein (L/P), yield, moisture at harvest (Moist), specific gravity (SpGr), and kernel hardness (Hard).

Inbred	Erot (x10)	Prot (x10)	Lys (x10)	L/P	Yield	Moist	SpGr (x100)	Hard (x10)
W32	-3.8	-4.7	-0.5	1.3	-7.2	-2.3	-1.0	-0.4
W64A	3.4	4.2	0.6	-1.0	9.8	0.5	2.5	13.7
RW153R	2.9	3.8	2.0	0.4	0.0	0.3	-1.7	-15.0
Oh43	-2.4	-3.2	-2.1	-0.7	-2.6	1.5	0.3	1.9
751015	0.7	-3.4	-2.1	-0.8	9.5	-0.5	0.8	-0.5
R168	0.7	0.5	0.5	0.3	-4.2	-0.3	-0.5	-4.5
Oh45	0.5	1.5	0.1	-0.4	-6.2	0.4	0.2	-0.6
Oh51A	-1.9	1.5	1.5	0.9	0.9	0.3	-0.4	5.7
R182	0.6	-1.4	-2.1	-1.5	4.1	-1.1	1.1	4.4
M14	3.4	-2.7	-1.6	-0.4	-1.0	-0.4	1.0	-0.3
B45	-1.2	-0.7	0.1	0.4	-3.0	2.1	-0.6	-2.1
B46	-2.7	4.7	3.6	1.6	0.1	-0.6	-1.5	-2.1

Table 20 continued.

Inbred	Erot (x10)	Prot (x10)	Lys (x10)	L/P	Yield	Moist	SpGr (x100)	Hard (x10)
Mo17	5.7	-0.2	0.2	0.3	-1.1	-1.2	-0.9	0.0
N28	-2.8	0.0	-1.6	-1.5	-1.5	0.5	4.5	4.4
N31	3.3	0.8	1.7	1.3	-0.1	-0.7	-2.9	-12.6
B37	-6.3	-0.5	-0.2	0.0	0.7	-2.7	-0.6	3.0
B57	3.1	-2.7	0.1	1.1	3.4	0.2	-1.2	-5.9
Va35	-5.3	0.8	-0.5	-0.8	0.1	-0.5	2.9	8.9
RVa36	-0.9	5.3	0.9	-1.0	-4.4	-0.5	-1.5	-1.1
CI64	3.1	-3.3	-0.5	0.7	0.8	0.8	-0.2	-2.1
C103	-6.5	2.1	1.4	0.5	-1.0	0.1	0.7	13.3
B65	10.8	0.8	-0.1	-0.3	-0.8	-2.7	-2.4	-2.9
RVa43	-4.3	-2.7	-1.3	-0.2	1.8	2.6	1.7	-10.5

Table 21. General combining abilities of the opaque-2 inbred lines used as males in the Design II experiment for ear rot (Erot), kernel rot (Krot), % protein (Prot), % lysine (Lys), % lysine-in-protein (L/P), yield, moisture at harvest (Moist), specific gravity (SpGr), and kernel hardness (Hard).

Inbred	Erot (x10)	Prot (x10)	Lys (x10)	L/P	Yield	Moist	SpGr (x100)	Hard (x10)
W37A	-1.1	-8.4	-4.1	-0.7	5.2	-0.2	2.8	1.3
A619	-2.4	-1.2	-0.6	0.0	-4.1	-0.2	-1.0	-4.9
A632	2.9	1.5	1.6	1.0	1.6	-0.6	-0.9	-1.6
B14A	0.7	8.3	3.1	-0.3	-2.6	-1.0	0.9	5.4
751039	-0.8	1.1	0.0	-0.4	2.6	-0.2	1.6	0.4
MS315	-1.6	-3.1	1.0	2.2	-8.5	-3.2	-2.8	-11.8
C123	1.1	-3.9	-2.5	-1.0	8.6	1.8	2.5	9.3
B66	1.2	6.1	1.5	-0.9	-2.8	1.6	-1.2	2.2
N6	-1.1	4.4	2.1	0.3	-7.6	2.9	1.0	13.9
R181B	4.5	-4.4	-2.0	-0.2	6.5	-2.2	-1.5	-14.9
R802	3.3	0.0	0.2	0.2	-3.4	0.2	0.7	3.0
751327	-6.6	0.0	-0.3	-0.3	4.5	-1.0	-0.2	-2.2

Table 21 continued.

Inbred	Erct (x10)	Prot (x10)	Lys (x10)	L/P	Yield	Moist	SpGr (x100)	Hard (x10)
751331	-2.6	0.1	0.2	0.1	4.0	-2.6	-1.1	-3.1
K41	-0.7	-0.7	-0.7	-0.4	2.1	1.7	1.2	-4.9
751440	2.0	1.1	1.2	0.7	5.8	0.1	-0.8	1.5
Mc20W	1.3	-0.4	-0.6	-0.4	11.9	0.8	-0.7	6.6
R803	-1.5	-0.4	-0.9	-0.7	-8.9	0.3	2.7	6.9
R75	-1.6	8.0	2.4	-0.7	-3.2	0.3	-0.4	2.2
751444	-3.8	2.3	-0.4	-1.2	14.8	-1.6	0.3	10.6
Oh7A	6.9	-9.9	-1.2	2.5	-2.6	1.0	-2.5	-19.8
751336	5.2	4.5	0.9	-0.8	7.4	2.5	-0.9	-5.4
751062	-4.3	-4.8	-1.6	0.4	3.2	-1.8	0.9	-1.6
751039	-4.0	-2.3	0.8	1.6	8.1	1.4	-0.9	-10.9
B59	9.6	1.7	0.4	-0.2	-3.2	-2.2	-2.1	-5.2
R109B	-6.6	1.2	-0.5	-0.9	-8.3	0.2	3.1	22.9

significant for all traits. Thus, comparisons of GCA's across sets should not be attempted.

The relative ear rot resistance of the lines employed in the Design II (Tables 18 and 19) compared well with their performance in the diallel (Table 13). The inbreds B37, B45, and N28 were rated as resistant, and B57 and C123 susceptible, in both experiments. The few discrepancies, notably B65 and N6, were partially due to the different hybrid combinations in the diallel and Design II. Ear rot ratings of half-sib families also compared favorably with their performance as inbreds, reported by Loesch et al. (76), although different inoculation techniques and rating scales were employed.

The hybrids generated by the Design II mating system were also evaluated in growth chamber experiments for resistance to seedling blight caused by F. moniliforme. The primary objective was to determine whether or not any relationship existed between seedling blight resistance and ear rot resistance. Significant differences were found among hybrids for all seedling traits (root, shoot, and total plant weight) when they were grown in infested or non-infested sand (Table 22). Mean squares associated with effects due to males (GCA), or due to females (GCA), were always greater than those associated with the effects due to the male by female interaction (SCA). This suggests that additive

Table 22. Analyses of variance of seedling root, shoot, and total plant weights grown in the presence or absence (control) of F. moniliforme inoculum.

		Mean squares								
		Control			Inoculated			% of Control		
Source	df	Root	Shoot	Plant	Root	Shoot	Plant	Root	Shoot	Plant
Set (S)	5	3537	2105	10166	68	636	1030	199	143	144
Rep/S	12	884	1446	4114	348	635	1747	1008	1055	978
Entry/S	82	549**	281**	1439**	34**	166**	323**	67**	246**	122**
Male (M)/S	16	365**	246**	935**	45**	159**	351**	115**	413**	220**
Female (F)/S	18	1430**	612**	3542**	78**	433**	807**	87**	208**	105*
M x F/S	48	280**	168**	818**	14*	67**	132**	44	204*	95*
Error	164	177	87	438	9	31	59	36	131	63

genetic variation (GCA) was more important than non-additive genetic variation (SCA) in inheritance of the seedling traits examined. The mean squares associated with the effects due to females were of greater magnitude than those due to males. This does not imply maternal differences, even though they would be expected in seedling traits, because in the mating design different lines are used as males and females (as contrasted to the diallel). This merely indicates there was more variation among the lines used as females than among lines used as males.

Seedling blight resistance was also evaluated by analyzing the root, shoot, and total plant weight of diseased seedlings expressed as the percent of control seedling weights. No significant correlations were found between any seedling trait and ear or kernel rot (Table 23). This lack of association was unfortunate, for a significant correlation would imply evaluation for ear rot resistance might be feasible in the seedling stage, thus greatly facilitating selection of resistant genotypes. This is in contrast to results from the field experiment, where ear rot and kernel rot were correlated with stand counts. The lack of any significant correlations suggests that mechanisms conferring resistance to F. moniliforme seedling blight were different from those conferring resistance to Fusarium ear rot. This is in agreement with Hooker (51) who found no correlation between

Table 23. Phenotypic correlations among ear and kernel rot, and seedling weights of plants grown in the presence or absence of F. moniliforme inoculum, from the Design II experiment.

	Control			Inoculated			% of Control		
	Root	Shoot	Plant	Root	Shoot	Plant	Root	Shoot	Plant
Ear Rot	-.06	-.03	-.05	-.13	-.14	-.14	-.07	-.15	-.14
Kernel Rot	.05	.07	.06	-.08	-.06	-.07	-.14	-.21	-.20

the inbred response to to seedling blight caused by D. zeae or G. zeae, and the inbred responce to ear rots caused by the same organisms.

These results suggest several alternatives in the attempt to develop high yielding maize with both resistance to Fusarium ear rot and good protein quality. First, the opaque-2 lines identified as having good GCA's for ear rot resistance generally were not those with the best GCA's for grain yield or lysine content, although B46, C103, Oh51A, and 751330 did exhibit good GCA's for all traits. Thus, improvement is needed in most public opaque-2 lines for either grain yield, ear rot resistance, or both. Ear rot resistance of opaque-2 lines presumably would be increased by the accumulation cf modifier genes that give vitreous kernels, or by the incorporation of the sugary-2 gene, both of which increase kernel hardness and kernel density. If Boling and Grogan's (5) estimate of the number of genes involved in ear rot resistance is reasonably correct, incorporation of resistance might be accomplished by a simple backcrossing program. The negative correlations between ear rot resistance and protein quality, and between protein quality and grain yield, however, suggest that the development of commercially-acceptible opaque-2 inbreds will be a slow process. Source populations with sufficient genetic variability, for ear rot resistance and other agronomic traits, have been identified (26, 124).

Heritability estimates and predicted gain per cycle were relatively high for ear rot resistance, but low for protein quality traits. Selection based on a composite of several traits, therefore, would be most successful, although time-consuming. A majority of the favorable modifier genes for protein quality and grain yield would be excluded initially. Thus, a breeding program in which desireable genes are gradually accumulated would be most effective. In the maize germplasm examined to date, most of the genetic variability for grain yield, protein quality, and ear rot resistance was additive (5, 26, 36, 124). Thus, selection programs which effectively utilize additive genetic variance, such as half-sib, full-sib, and S-1 recurrent selection, appear to be the most efficient for intrapopulation improvement of these traits.

SUMMARY AND CONCLUSIONS

Ear rots in general, and specifically the ear and kernel rots incited by Fusarium moniliforme, are a major factor limiting the utilization of opaque-2 (high-lysine) maize.

Forty-seven opaque-2 families, grown at one location, were examined to determine the predominant fungi causing ear rot. Ear rot, measured as percent kernel infection with the deep-freezing blotter technique, was caused primarily by F. moniliforme. Other major fungal pathogens were Aspergillus spp., Penicillium spp., and Rhizopus sp. Kernel infection by Fusarium sp. or other fungal genera was not significantly correlated with either percent germination or abnormal seedlings. The procedure used to determine kernel infection may not be sufficient in itself to predict field performance of infected seed lots.

Two popular methods of artificial inoculation were tested for evaluation of ear rot resistance. The toothpick-ear puncture method was more reliable, compared to the silk spray method, for distinguishing F. moniliforme resistant and susceptible genotypes. In addition, the relative ranking of the genotypes for ear rot resistance differed appreciably between the two inoculation methods. Estimation of the percent rotted area on a linear scale, followed by logarithmic trans-

formation, was found to be an efficient means of rating ear rot. Actual measurement of the diseased area was possible when the ears were inoculated with the ear puncture method. This rating was less subject to bias than the subjective estimation of ear rot. Ear rot development, as determined by incubating the ears in moisture chambers, reached a maximum level 6 weeks after inoculation. This would imply that evaluation of ear rot resistance could be done prior to harvest and thus save considerable time as only the selected genotypes need be harvested.

Kernels from a seed lot with a high percentage of F. moniliforme infected kernels were grown to maturity in two growth chamber experiments. Under conditions which prevented seed- and soil-borne F. moniliforme from becoming air-borne, infected kernels produced systemically-infected plants with subsequent ear infection. Infected seed may result in ear rot, although wind-blown spores probably constitute the major inoculum source for ear rot in the field.

Forty-five opaque-2 hybrids and their reciprocals, generated from a diallel mating design of 10 public opaque-2 inbred lines, were evaluated for resistance to F. moniliforme ear rot. Significant differences in ear rot resistance were found among the hybrids and among the general combining ability (GCA) effects. Specific combining ability (SCA), maternal, and reciprocal effects were not significant. Thus,

in the material examined, additive genetic variation (GCA) was important, while non-additive genetic variation (SCA) was not important in inheritance of ear rot resistance. The opaque-2 inbreds B37, B45, and B65 displayed the best GCA's, and B57, C123, and N6 the poorest GCA's for ear rot resistance.

Ninety-five opaque-2 hybrids, generated with a Design II mating plan employing 48 opaque-2 inbred lines, were evaluated for F. moniliforme ear rot resistance, and agronomic, protein quality, and kernel traits. Significant differences were found among hybrid entries for all traits. The effects due to males (GCA), females (GCA), and the male by female interaction (SCA) were statistically significant for all traits except stand. Additive genetic variation (GCA) was two to ten times larger than non-additive genetic variation (SCA) for all traits in the material examined.

Ear rot severity was negatively correlated with stand, tassel date, kernel density, kernel hardness, percent moisture at harvest, and yield. Percent lysine and percent lysine-in-protein were positively correlated with ear rot severity, while there was no significant correlation between percent protein and ear rot severity. Pericarp thickness of mature kernels had no bearing on ear rot resistance. Differences in kernel characteristics (i.e. lower kernel density and lower kernel hardness), resulting from increased lysine

content and altered starch metabolism, probably accounted for increased ear rot severity more than differences in lysine content per se. Ear rot resistance was not correlated with resistance to seedling blight caused by F. moniliforme, implying that selection for ear rot resistnce cannot be accomplished at the seedling stage.

Inbred lines conferring good ear rot resistance generally imparted less than optimum protein and lysine levels. The half-sib families of B45, B46, C103, N6, Oh51A, 751327, and 751331, however, exhibited good GCA's for ear rot resistance without much decrease in percent protein or lysine. Several widely used opaque-2 inbreds, including A632, B59, B65, C123, Mo17, Oh7A, R181B, and W64A, displayed poor ear rot resistance in hybrid combination.

The unfavorable correlations of ear rot resistance with lysine content, yield, and other agronomic traits suggest that the development of improved inbred lines will be difficult if selection is based on one trait alone. Selection based on several traits, including ear rot resistance, yield, and protein quality, would be more successful. This process, involving the gradual accumulation of favorable modifier genes for grain yield and protein quality, would not be expected to bear results in a short period of time. As additive gene action is of more importance than non-additive gene action in inheritance of ear rot resistance and all other

traits, a selection program which effectively exploits additive gene action, such as half-sib, full-sib, or S-1 recurrent selection, would presumably be most effective for simultaneous improvement of these traits.

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APPENDIX

Table 23. Entry means from the Design II experiment for stand, tassel date (Tas), ear rot (Erot), kernel rot (Krot), kernel volume (Vol), kernel weight (Wt), kernel specific gravity (SpGr), moisture at harvest (Moist), kernel hardness (Hard), abgerminal pericarp thickness (PA), germinal pericarp thickness (PG), average pericarp thickness (PAV), protein (Prot), lysine (Lys), lysine in protein (L/P), and yield.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
W37A	W32	18.5	3.5	3.79	4.15	56	61.7	1.10	17.4
W37A	W64A	20.8	6.8	5.80	5.29	63	69.9	1.10	18.9
W37A	RW153R	20.0	4.0	5.53	5.30	74	80.3	1.09	19.2
W37A	Oh43	24.5	4.8	3.51	4.52	68	73.4	1.09	20.5
A619	W32	19.3	1.0	3.21	4.90	62	63.3	1.03	16.6
A619	W64A	23.0	4.0	3.88	4.60	70	77.8	1.11	21.0
A619	RW153R	21.8	1.3	4.98	5.29	83	85.6	1.04	18.8
A619	Oh43	17.5	7.5	6.06	6.10	70	7.4	1.06	19.4
A632	W32	22.0	2.5	5.16	5.31	56	58.8	1.05	16.2
A632	W64A	21.0	6.5	5.29	5.45	58	63.4	1.09	19.5
A632	RW153R	26.8	3.8	5.01	5.48	73	75.4	1.03	18.5
A632	Oh43	20.0	6.3	4.77	5.12	65	69.7	1.07	19.7
B14A	W32	20.8	2.8	5.40	5.43	54	56.7	1.05	17.2
B14A	W64A	23.0	8.3	5.47	5.50	58	61.7	1.07	19.1
B14A	RW153R	19.0	5.3	4.73	5.21	69	72.0	1.04	21.0
B14A	Oh43	19.3	8.0	3.77	4.38	59	62.4	1.07	22.9
Set	Mean	21.1	4.8	4.77	5.84	65	69.1	1.07	19.1

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
W37A	W32	12.5	73	53	63	10.0	.40	4.02	33.0
W37A	W64A	13.6	70	56	63	10.7	.42	3.91	48.8
W37A	RW153R	11.0	60	43	52	10.7	.44	4.08	42.0
W37A	Oh43	12.6	74	50	62	9.6	.38	3.93	42.6
A619	W32	11.8	68	49	58	10.5	.44	4.22	26.4
A619	W64A	14.1	65	49	57	11.5	.45	3.88	52.0
A619	RW153R	10.1	52	42	47	11.1	.45	4.05	32.1
A619	Oh43	11.1	59	46	53	10.8	.44	4.07	18.8
A632	W32	11.7	76	52	64	10.5	.45	4.32	34.2
A632	W64A	13.2	76	57	66	11.6	.47	4.08	40.8
A632	RW153R	10.7	65	50	58	11.7	.49	4.18	37.7
A632	Oh43	12.9	75	52	63	11.1	.45	4.02	39.4
B14A	W32	13.0	88	56	72	11.5	.48	4.16	23.5
B14A	W64A	13.7	79	62	71	12.3	.49	3.97	43.4
B14A	RW153R	11.3	73	56	64	12.3	.50	4.07	33.9
B14A	Oh43	13.2	79	59	69	11.6	.45	3.90	34.5
Set	Mean	12.3	71	52	61	11.1	.45	4.05	36.5

Table 23 continued.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
751039	751015	22.5	9.3	3.63	4.29	65	73.0	1.13	19.3
751039	R168	24.0	8.5	3.39	4.39	51	57.8	1.14	18.0
751039	Oh45	25.8	7.0	3.35	4.05	63	72.3	1.15	21.2
751039	Oh51A	26.0	7.8	3.91	4.36	61	68.5	1.12	20.5
MS315	751015	24.3	4.5	3.72	4.86	57	62.2	1.09	15.9
MS315	R168	22.5	3.0	3.86	4.98	48	52.1	1.08	16.2
MS315	Oh45	23.0	1.5	3.03	4.73	61	67.2	1.10	17.3
MS315	Oh51A	23.5	2.5	3.34	4.31	55	60.4	1.09	17.5
C123	751015	19.5	9.8	3.91	4.49	73	84.5	1.17	22.1
C123	R168	22.5	8.8	3.91	4.64	63	71.0	1.14	21.4
C123	Oh45	24.5	7.0	4.11	5.02	76	85.3	1.13	21.7
C123	Oh51A	22.8	7.5	3.10	4.28	70	80.0	1.14	22.0
B66	751015	19.5	9.0	3.61	4.41	66	73.7	1.12	20.7
B66	R168	21.0	7.0	3.69	4.72	59	64.3	1.10	23.1
B66	Oh45	24.5	6.5	4.30	5.23	69	75.7	1.10	21.3
B66	Oh51A	23.0	7.0	3.46	4.36	65	72.1	1.11	21.2
Set	Mean	23.0	6.7	3.65	5.26	62	70.0	1.12	20.0

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
751039	751015	14.3	47	46	47	10.6	.41	3.84	57.0
751039	R168	14.1	44	41	42	10.4	.41	3.92	38.8
751039	Oh45	14.9	42	35	39	10.1	.40	3.98	51.7
751039	Oh51A	15.5	48	45	47	10.9	.43	3.90	48.9
MS315	751015	13.3	70	53	62	9.7	.41	4.19	47.2
MS315	R168	13.2	65	45	55	10.1	.43	4.24	29.3
MS315	Oh45	13.5	66	47	57	10.3	.41	4.00	35.3
MS315	Oh51A	14.0	67	52	59	10.2	.43	4.26	40.5
C123	751015	15.7	69	61	65	9.3	.35	3.73	65.8
C123	R168	15.0	66	55	60	10.3	.39	3.75	56.7
C123	Oh45	14.7	69	55	62	10.5	.40	3.79	41.1
C123	Oh51A	17.0	72	64	68	9.9	.41	4.13	57.2
B66	751015	15.2	72	59	66	10.6	.40	3.73	54.2
B66	R168	14.5	60	49	55	10.9	.44	4.01	44.5
B66	Oh45	15.3	68	50	58	11.3	.44	3.86	33.2
B66	Oh51A	14.5	58	54	56	11.2	.43	3.85	43.3
Set	Mean	14.7	61	51	56	10.4	.41	3.95	46.5

Table 23 continued.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
N6	R182	25.0	9.0	3.83	4.52	75	83.6	1.11	23.4
N6	M14	25.0	10.8	3.40	4.85	69	77.5	1.12	25.3
N6	B45	25.0	11.0	4.34	5.44	68	74.6	1.10	27.2
N6	B46	22.5	10.5	3.33	4.68	68	74.1	1.09	23.3
R181B	R182	24.5	9.3	4.56	4.13	70	76.2	1.09	19.2
R181B	M14	23.8	10.5	5.09	4.90	67	72.9	1.10	18.2
R181B	B45	22.0	12.3	3.81	4.23	74	79.6	1.08	22.2
R181B	B46	24.5	10.3	3.70	3.78	75	79.0	1.06	19.2
R802	R182	23.0	9.0	4.25	4.81	63	70.1	1.11	21.3
R802	M14	24.3	11.3	4.67	5.75	69	76.4	1.10	22.5
R802	B45	20.0	9.8	3.54	4.97	72	79.0	1.10	22.7
R802	B46	23.0	10.5	4.21	5.64	68	74.1	1.09	21.9
751327	R182	24.3	10.5	2.94	4.04	72	79.7	1.11	19.2
751327	M14	26.0	11.0	3.54	5.28	70	76.9	1.10	20.0
751327	B45	24.8	11.5	3.20	5.07	70	75.7	1.08	23.9
751327	B46	26.0	10.3	3.03	4.19	68	73.9	1.09	20.6
Set	Mean	24.0	10.5	3.84	5.15	70	76.5	1.10	21.9

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
N6	R182	14.7	73	59	66	10.5	.42	3.98	38.1
N6	M14	16.7	55	51	53	10.7	.43	4.03	38.5
N6	B45	15.1	48	51	49	10.6	.46	4.30	24.7
N6	B46	14.3	57	55	56	11.3	.48	4.29	36.9
R181B	R182	12.8	56	44	50	9.7	.38	3.91	46.5
R181B	B14	11.6	47	37	42	9.1	.38	4.22	47.7
R181B	B45	12.6	56	46	51	10.1	.41	4.00	50.5
R181B	B46	12.2	61	47	54	10.6	.46	4.30	49.9
B802	R182	15.1	79	57	68	10.3	.42	4.08	43.9
R802	M14	11.4	58	49	53	10.3	.41	4.00	33.1
R802	B45	13.9	55	51	56	10.1	.42	4.15	41.7
R802	B46	14.1	64	54	59	10.5	.45	4.33	36.2
751327	R182	14.4	72	56	64	10.3	.40	3.93	56.2
751327	M14	13.5	48	41	44	10.1	.41	4.08	45.4
751327	B45	12.8	64	57	61	10.1	.42	4.20	39.3
751327	B46	13.8	64	55	59	10.8	.45	4.19	45.6
Set	Mean	13.8	60	51	55	10.3	.43	4.12	42.1

Table 23 continued.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
751331	Mo17	24.3	10.5	3.70	4.01	82	92.8	1.14	20.5
751331	N28	22.0	11.5	3.77	3.89	79	91.2	1.16	26.8
751331	N31	19.8	11.3	3.01	3.40	83	93.4	1.13	22.8
751331	B37	21.5	9.8	2.98	3.57	75	85.5	1.14	19.8
K41	Mo17	23.8	10.8	4.15	4.21	81	95.1	1.17	25.6
K41	N28	24.0	11.5	3.05	3.16	71	85.6	1.21	32.7
K41	N31	21.0	9.3	4.21	4.20	90	10.2	1.13	24.1
K41	B37	24.8	11.3	2.79	2.83	81	92.1	1.14	24.7
751440	Mo17	24.0	11.5	4.03	4.20	79	8.9	1.13	24.7
751440	N28	24.0	11.5	3.55	4.23	69	82.0	1.20	26.0
751440	N31	23.3	9.3	4.10	4.93	81	91.2	1.13	24.9
751440	B37	22.0	8.9	3.19	4.03	74	84.5	1.15	20.8
Mo20W	Mo17	23.8	9.5	4.91	4.85	83	93.6	1.13	24.9
Mo20W	N28	24.5	11.5	3.01	4.35	63	76.0	1.22	29.7
Mo20W	N31	23.3	9.3	4.10	4.93	81	91.2	1.13	24.9
Mo20W	B37	23.0	10.3	3.01	3.99	68	79.3	1.16	24.3
Set	Mean	23.0	10.5	3.62	4.71	77	89.3	1.16	25.1

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
751331	Mo17	14.6	73	59	66	10.4	.39	3.74	50.2
751331	N28	14.7	71	65	68	10.5	.39	3.70	56.8
751331	N31	14.2	81	67	74	10.7	.41	3.83	58.7
751331	B37	15.1	84	67	76	10.4	.40	3.79	49.6
K41	Mo17	15.1	73	55	64	10.3	.38	3.70	53.2
K41	N28	15.6	74	60	67	10.3	.36	3.51	47.7
K41	N31	12.6	84	55	70	10.3	.41	3.94	55.1
K41	B37	14.7	92	63	78	10.8	.40	3.70	52.0
751440	Mo17	15.0	79	59	69	10.8	.41	3.83	50.4
751440	N28	14.1	77	57	67	10.8	.44	4.03	56.7
751440	N31	14.1	74	61	67	10.5	.39	3.72	28.5
751440	B37	15.7	80	67	73	10.3	.39	3.75	55.8
Mo20W	Mo17	15.1	72	62	67	10.4	.40	3.85	41.6
Mo20W	N28	17.6	70	75	72	10.7	.37	3.50	37.2
Mo20W	N31	14.1	74	61	67	10.5	.39	3.72	28.5
Mo20W	B37	15.7	78	68	73	10.2	.39	3.77	44.8
Set	Mean	15.0	77	62	70	10.5	.39	3.75	49.9

Table 23 continued.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
R803	B57	18.8	11.5	3.96	4.48	87	100.7	1.16	29.3
R803	Va35	18.3	13.0	4.54	4.94	88	101.7	1.16	26.8
R803	RVa36	19.3	13.0	3.79	4.17	77	87.2	1.13	26.3
R803	CI64	19.3	12.8	4.87	4.73	88	102.4	1.17	26.8
R75	B57	24.3	11.0	5.01	5.33	76	83.7	1.10	26.4
R75	Va35	21.3	11.3	4.17	4.78	80	92.3	1.15	27.4
R75	RVa36	22.3	10.3	3.93	4.50	77	86.0	1.11	27.1
R75	CI64	21.0	11.5	3.98	4.26	74	83.1	1.13	28.4
751444	B57	19.0	10.3	4.57	5.06	75	84.1	1.12	27.0
751444	Va35	22.3	11.8	3.12	4.23	80	93.3	1.17	23.7
751444	RVa36	24.8	11.0	4.12	4.15	78	87.4	1.12	26.3
751444	CI64	21.8	11.0	4.41	4.39	78	87.1	1.11	25.1
Oh7A	B57	21.5	12.0	5.44	5.40	76	82.7	1.08	26.4
Oh7A	Va35	22.8	12.8	3.79	4.67	93	106.1	1.14	28.4
Oh7A	RVa36	19.5	11.0	5.55	5.07	77	84.0	1.09	26.4
Oh7A	CI64	22.3	14.0	5.73	5.37	85	92.4	1.09	31.1
Set	Mean	21.2	11.8	4.44	5.41	80	90.9	1.13	27.0

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
R803	B57	15.3	81	65	73	10.3	.39	3.80	47.7
R803	Va35	14.5	86	68	77	10.7	.39	3.64	35.4
R803	RVa36	14.5	78	59	68	11.7	.42	3.55	16.2
R803	CI64	15.1	73	61	67	10.1	.39	3.89	44.7
R75	B57	13.6	85	68	77	11.1	.44	3.93	46.2
R75	Va35	14.9	88	71	79	11.7	.42	3.63	40.5
R75	RVa36	14.7	78	58	68	12.1	.43	3.57	37.4
R75	CI64	14.3	78	49	64	11.2	.42	3.77	42.3
751444	B57	14.7	68	54	61	11.1	.41	3.74	50.8
751444	Va35	17.6	69	50	59	11.1	.39	3.48	64.7
751444	RVa36	14.4	65	41	53	11.2	.41	3.68	42.0
751444	CI64	14.3	59	41	50	10.5	.40	3.77	61.2
Oh7A	B57	10.8	63	57	60	9.4	.39	4.12	48.7
Oh7A	Va35	13.2	68	64	66	9.8	.40	4.10	39.6
Oh7A	RVa36	12.7	52	47	49	10.0	.40	3.95	46.4
Oh7A	CI64	12.2	57	53	55	9.8	.39	3.99	34.7
Set	Mean	14.2	72	57	64	10.7	.40	3.79	44.9

Table 23 continued.

Male	Female	Stand	Tas	Erot	Krot	Vol	Wt	SpGr	Moist
751336	C103	21.0	10.3	3.55	3.97	89	97.8	1.10	27.0
751336	B65	17.8	11.3	6.62	6.07	80	87.2	1.09	22.8
751336	RVa43	22.8	11.3	3.79	3.77	77	86.3	1.13	29.1
751062	C103	19.8	9.5	2.95	4.81	92	103.7	1.12	24.1
751062	B65	19.8	9.8	4.85	4.94	99	108.1	1.10	20.4
751062	RVa43	24.0	9.3	3.30	3.70	87	99.4	1.15	24.2
751030	C103	24.5	10.0	3.12	4.14	79	88.8	1.12	25.5
751030	B65	23.5	11.8	4.61	4.98	86	92.4	1.07	22.0
751030	RVa43	20.5	11.5	3.46	3.33	80	88.8	1.12	28.1
B59	C103	23.5	9.3	4.54	5.05	78	85.9	1.10	21.7
B59	B65	22.3	10.8	5.57	5.73	79	86.3	1.09	19.0
B59	RVa43	21.0	9.8	5.17	4.99	78	84.6	1.09	24.1
R109B	C103	26.3	9.0	3.25	3.74	71	82.1	1.16	24.1
R109B	B65	22.3	9.5	4.41	4.49	78	86.4	1.10	21.6
R109B	RVa43	22.5	10.3	2.75	3.48	65	75.7	1.17	26.2
Set	Mean	22.1	10.2	4.13	5.16	81	90.2	1.11	23.8

Male	Female	Hard	PA	PG	PAV	Prot	Lys	L/P	Yield
751336	C103	14.0	65	61	63	10.9	.44	3.99	53.1
751336	B65	12.8	66	64	65	11.0	.43	3.95	51.9
751336	RVa43	12.1	44	51	47	10.7	.41	3.78	55.9
751062	C103	13.7	82	69	75	10.1	.41	4.08	49.6
751062	B65	13.5	78	76	77	10.2	.41	4.02	44.9
751062	RVa43	12.8	55	57	56	9.5	.38	3.98	53.9
751030	C103	13.9	92	69	81	10.5	.44	4.22	45.0
751030	B65	11.9	85	69	77	10.3	.43	4.15	49.2
751030	RVa43	11.4	68	60	64	9.8	.40	4.07	46.9
B59	C103	14.2	89	64	77	11.0	.44	3.95	37.6
B59	B65	13.4	65	58	61	10.3	.40	3.87	40.4
B59	RVa43	11.3	61	57	59	10.4	.43	4.09	51.2
R109B	C103	18.2	95	65	80	10.6	.42	3.95	40.8
R109B	B65	14.5	74	63	69	10.7	.41	3.80	40.6
R109B	RVa43	14.6	63	53	58	10.3	.41	3.95	32.4
Set	Mean	13.5	72	62	67	10.4	.42	3.99	46.2